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PRINCIPAL INVESTIGATOR: John R. Harsh

PI ADDRESS: University of Southern Mississippi
Hattiesburg, MS 39406-9371

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This report describes progress in a program of research concerned with whether event-related brain potentials (ERPs) provide a reliable, valid, and practical way of predicting performance. Two objectives of the program were to develop the capacity to measure, analyze, and interpret ERPs and to demonstrate that ERPs are sensitive to factors influencing performance. Experiments are described that show ERP variation in studies of orienting responses, habituation, and Pavlovian conditioning. Additional experiments show ERP changes in relation to time-of-day and ultradian variation in performance. A final experiment describes the relationship between ERP and performance changes during the wake/sleep transition. These experiments encourage the view that ERPs are closely related to both arousal and cognitive factors influencing performance. Keywords: —

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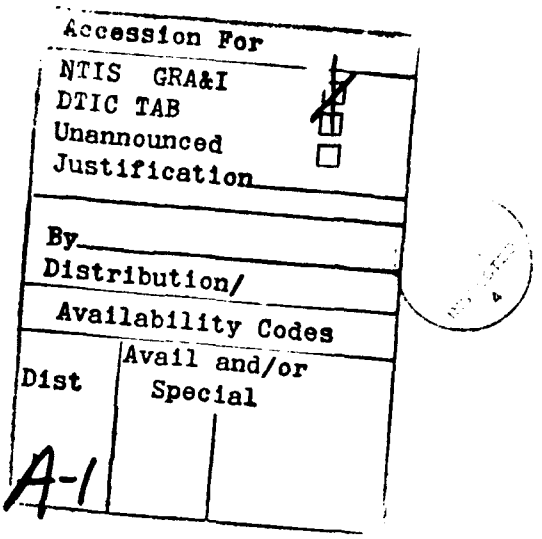

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John Hank 6-17-90

PI Signature Date

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5.0 INTRODUCTION

THE PROBLEM

Increases in technological sophistication in civilian and military work settings has resulted in greater demands being placed on human operators of man-machine systems. Often, machines respond more quickly than humans and perform a greater number of complex functions. However, such systems require surveillance by alert human monitors. The latter has resulted in a greater need to understand the factors which affect the performance of human monitors and has created a greater need to assess their performance readiness, particularly under conditions that might reduce readiness (e.g. sleep loss fatigue, boredom, hypothermia, hyperthermia, exposure to chemical agents, etc.).

In the past, researchers have used a variety of measures to infer central states related to performance readiness. The measures generally used were blood and urine composition, heart rate, galvanic skin response, electromyography, and others. Inferences based on these measures, however, have been of limited value in predicting and understanding performance changes. One likely reason for the lack of success is that these measures deal with peripheral physiological systems which are too distant from central processes to permit valid inferences. The purpose of the present research is to assess the usefulness of event-related brain potentials (ERPS) which are considered to be more closely related to central processes.

Event-related potentials are brain potentials that can be recorded in response to disrete stimuli. When measured under appropriate conditons, some ERP components are related to the physical characteristics while others are related to the psychological characteristics of stimuli. Recent technological developments have resulted in reliable and efficient procedures for recording, measurement and quantification of this activity.

Evoked potentials have been found to be related to performance on tasks involving stimulus detection, discrimination, and decision making. Further, they are thought to provide neurophysiological correlates of central states such as attention, information processing, and allocation of processing resources.

The research of concern here is about whether ERP measures may provide a reliable and practical way of predicting performance changes resulting from the effects of environmental, task, and field conditions. We have completed one major study involving ERPs and sleep deprivation (U.S. Army contract titled Auditory Evoked Potentials as a Function of Sleep Deprivation and Recovery Sleep"). The latter focussed on identifying fundamental relationships that exist between ERP measures, different levels of sleep deprivation and recovery sleep, and performance on several tasks involving psychological functioning and psychomotor performance. Sleep deprivation was chosen as a laboratory manipulation because: 1) previous research indicated that evoked potentials change with sleep and sleepiness (e.g., Gauthier and Gottesman, 1983); 2) it is an important, yet simple variable to

quantify and to vary systematically; 3) it enters into relationships with many other variables; 4) it has high inherent interest in military and civilian work settings (e.g., Naitoh and Townsend, 1970).

RESULTS OF PREVIOUS WORK

We found several interesting relationships in the research conducted under our first U.S. Army contract. These relationships over 48-hrs of sleep deprivation can be summarized as follows: 1) sleep deprivation results in performance degradation; 2) the degradation in performance is marked by circadian effects; 3) sleep deprivation also has a marked effect on cortical evoked response measures 4) the circadian variation is also apparent in the cortical evoked responses recorded during the deprivation period); 5) later (N2, P3) components of evoked responses covary with changes in performance associated with sleep deprivation and circadian variation; 6) earlier (P1, N1, P2) evoked potential components covary with individual differences in performance throughout the deprivation period; and 7) the predictive relationships between ERPs and performance appear stronger for tasks involving a rate measure than for tasks not involving a rate measure.

One reason that our earlier findings are potentially important for prediction of changes in performance is that some of the relationships involved N2 deflection of the evoked potential. N2 can be recorded with minimal intrusion. The sensitivity of P3, for example, depends on subjects carefully attending to the eliciting stimulus, N2 may not. Thus N2 across sleep deprivation conditions may be a particularly useful predictor of performance degradation.

Another reason why the findings may be important is that N1, P3, and N2 correlated with individual differences in performance. This finding adds to the evidence that evoked potentials provide a basis for classification/selection of individuals in some performance settings (cf. Lewis, 1983; Wilson and O'Donnell, 1986).

A further reason that the results may be important relates to the finding that the evoked potentials were more clearly related to the performance measures involving rate as a response dimension. ERP measures may be selectively related to the attentional, perceptual, and processing activities underlying rate measures of performance. It is particularly important that a better understanding of this relationship be developed so that the usefulness of evoked potentials in predicting performance degradation can be assessed.

PROPOSED WORK

The research proposed under our current contract is concerned with the replication and extension of the findings of our initial experiment. The goals are as follows. 1) to replicate and extend the findings noted above and assess the extent to which ERPs can be used to provide a greater understanding of the attentional, perceptual, and processing changes underlying performance degradation in a stressful

environment; 2) to develop a predictive model (see below) between the different components of evoked responses and performance degradation; 3) to identify the maximum lead-time in which evoked responses may predict performance changes; 4) to assess ways of enhancing the relationship between changes in evoked responses and changes in performance; 5) to evaluate techniques used by others to measure central nervous system activity; and 6) to assess the extent to which evoked responses can be used to identify those individuals who will perform well on selected tasks from those who will not perform well.

RESEARCH PLAN

The plan described in our current contract for achieving the goals included a three-phase program. During Phase I (Year 1), the general objectives were to develop further our capability to measure, analyze, and interpret event-related potentials and to refine our existing battery of performance measures. The objectives of Phase II (Year 2) were to replicate and extend our earlier findings and demonstrate that evoked-response measures are at least as sensitive to conditions degrading performance as more conventional measures. Phase III objectives are to examine correspondance between ERPs and performance during long-term sleep deprivation.

RESEARCH PROGRESS

Software Development. Considerable progress in achieving the goals described above was made in the first half of the project period. A major accomplishment for our laboratories was the development of software for the organization, display, and evaluation of ERPs. This program called "EVAL" is unique in the research community in that it was written for a PC rather than a main frame computer. It offers the ability to filter, correct for artifact, and display and analyze data on a sweep-by-sweep or averaged basis.

Research. Several experiments were completed during the first half of the project period. Several additional experiments were intiated. The initiated but not completed experiments are described in the quarterly reports. The section below describes completed experiments.

6.0 BODY

6.1 Experiment 1 - Habituation of the P300 to Target Stimuli: Are Arousal and Attention Factors?

This research focusses on the decrement in amplitude of the P300 to target stimuli under conditions of repeated testing (i.e., habituation). It is generally assumed that the P300 to task-related stimuli (targets) does not habituate even though habituation to non-task related stimuli (non-targets) occurs rapidly (e.g., Courchesne, Courchesne, & Hillyard, 1978; Megela & Tyler, 1979). However, recent findings have revealed that a decrement in P300 amplitude to targets can also occur (Wesensten, Badia, & Harsh, 1988). The findings regarding targets found a 40% decrement in P300 amplitude with repeated testing. Why and under what conditions does this P300 index of cognitive processing habituate?

Experiment 1a provides a detailed assessment of P300 habituation and dishabituation (recovery). Several conditions under which dishabituation might occur were examined. The latter were chosen based on studies (e.g., Badia & DeFran, 1970; Thompson & Spencer, 1966) showing habituation and dishabituation using other response measures (i.e., skin conductance response [SCR]). It was assumed that conditions resulting in recovery of the SCR will affect the P300 in a similar manner. Thus, for one condition of Experiment 1a we introduced a stimulus change during testing to determine whether an habituated P300 will recover. For a second condition, the incentive effect of information was assessed. There are data which indicate that giving subjects information about when the experimental session is nearing an end results in enhanced performance (Haslam, 1983).

Experiment 1a

A standard oddball paradigm was used and we focussed on the pattern of habituation across blocks of 35 targets. Following five blocks of testing, four conditions were imposed: a) Group 1, no change; b) Group 2, reversal of target and non-target tones; c) Group 3, information (Knowledge) that the last block of testing was to occur; d) Group 4, both b and c.

Method

Subjects and Procedure Forty subjects (freshman college students, 10 male, 30 female) were assigned randomly to the four conditions. Evoked potentials were recorded using Grass amplifiers and electrodes. Tones were presented via headphones at 80 dB SPL. Probability of targets (1200 Hz) was 0.2, whereas probability of non-targets (1000 Hz) was 0.80. The interstimulus interval was 1500 ms, digitizing was at 280 Hz with a signal averaging window of 810 ms. Six blocks of testing were given, each containing 35 targets and about 160 non-targets. The interblock interval was 20 s. The first 5 blocks were identical for all subjects and the treatment was introduced on Block 6.

Results

Habituation Figure 1 shows the diminution in P300 amplitude across Blocks 1-5. The decrease becomes very apparent when comparisons are made between Block 1 and Block 5; amplitude decreased from 11.3 uV on Block 1 to 5.4 uV on Block 5. Statistical analysis revealed that the main effect for Block was significant, $F(4,144)=22.7$, $p<.01$. The analysis of P300 latency revealed the same effect in that latencies increased significantly ($p<.01$).

Treatment Effects (Dishabituation) Figure 1 also shows the change in amplitude (recovery) from Block 5 to Block 6 for the four conditions. A significant main effect for Groups was found, $F(3,36)=5.4$, $p<.05$. As shown, a substantial increase in P300 amplitude occurred only for the Knowledge Group and the Knowledge & Reversal Group. The Reversal Group did not differ from the Control group.

Within Block Analysis Figure 2 depicts the data of the "Knowledge" groups somewhat differently. For Figure 2 each of the 7 points within a block represents an average across 5 targets instead of across 35 targets as shown earlier. The total number of targets and non-targets remain the same. One advantage of averaging over blocks of 5 targets is that it permits one to observe changes that may occur within the larger block of 35 targets. The amplitudes at the beginning of each block were generally higher and then show a decrease within a block. Each block shows the same pattern: amplitude recovery at the beginning of the block followed immediately by a diminution in amplitude for the remainder of the block. This sub-blocks analysis was statistically significant ($p<.05$).

Discussion

Both habituation and dishabituation of P300 amplitude were clearly documented. Two conditions gave rise to dishabituation. One was the treatment condition of "Knowledge" and the other was the interblock interval (Within Block analysis). Both may be related. Why are the amplitudes higher at the beginning of a block? Why do they then decrease? Several events occurred between blocks. During this time the participant was asked the number of targets presented. Their answers were generally very accurate. After a moment to relax they were instructed that another series of tones was to begin and that they should again count the targets and press the switch. These events may have refocussed them on the task and may have been responsible for the increase in amplitude at the beginning of each block. Post experimental interviews provided some support for the latter. Subjects reported that the task was very easy, required little effort, and that it was very boring. They reported being more into the task at the beginning of the block. As stimuli continued to be presented, however, other factors began to exert an effect. Subjects reported growing bored, drowsy and thoughts unrelated to the task intruded and increased in frequency.

How do we interpret these observations? Is the reduction in amplitude across blocks due to a reduction in attention to the

target task, i.e., intrusion of competing thoughts? In effect, subjects were performing two concurrent task--the target task and unrelated intrusive thoughts. There are data showing that requiring a concurrent task (mental arithmetic, tracking a different target) while instructed to count targets results in a decrease in P300 amplitude. Another factor affecting P300 amplitude could be arousal level. Could the decrease in amplitude be related to a decrease in arousal/alertness? Subjects did report being very bored and, at times, drowsy. We know that arousal level can have powerful effects. For example, in sleep deprivation studies of 48 to 72 hours, performance deteriorates considerably. However, if subjects are informed that the deprivation period is about to end subjects are aroused and performance level makes a remarkable recovery.

Obviously habituation was reversed by the information condition. However, these findings are compatible with both the "arousal" interpretation and the "attention" interpretation. One or both factors may be important. Our subjects reported higher arousal/alertness after being given the "end-of-session" information and also being more attentive to the task because they knew that it was their last effort. Thus, an increase in general arousal and an increase in attention to the task occurred for the knowledge groups. We attempted to separate these two factors in our next experiment.

Experiment 1b

For our next study we measured arousal levels using tonic skin conductance level (SCL) as the measure. Our interest was in contrasting an interpretation focussing on arousal with an interpretation focussing on attention to the task. Again, the oddball task was used. Six blocks of testing were given and SCLs were measured from the first block to the end of the sixth block. We assumed that the greater the SCL, the higher the arousal level.

Method

Subjects and Procedure Twenty undergraduate female subjects were assigned randomly to two groups. The first three blocks were the same for all groups and the treatment was introduced at the beginning of Block 4. Thus, we expected that P300 amplitude would show a decrease across the first three blocks (i.e., habituation). We assessed whether arousal also declined. For Group 1, subjects were instructed at the beginning of Blocks 4, 5, and 6 to "Stand" upright from their sitting position for a few seconds ("Stand" condition) and to stretch fully. We assumed that doing so would combat any drowsiness and boredom and would increase arousal level as indexed by the SCL. Group 2 subjects were instructed not only to "Stand" and "Stretch", but they were also informed that they would receive, at the end of each block, $1.00 for correct responding. We assumed that money would focus their attention on the task.

Results and Discussion

Figure 3 shows the results of these manipulations. The decrease in P300 amplitude across the first 3 blocks

(habituation) was significant, $F(2,38)=18.2$, $p<.01$. Figure 3 also shows that tonic skin conductance levels dropped sharply $F(2,38)=31.8$, $p<.01$) over Blocks 1-3 and that this occurred about equally for both groups ($F<1$). On Block 4 the treatment conditions were introduced. Again, it is clear that arousal (tonic SC levels) increased to similar levels for both conditions across Blocks 4, 5, and 6 ($F<1$) yet only the "attention" condition was effective in increasing P300 amplitudes (dishabituation). Obviously since SCL was essentially the same for both conditions, simply increasing arousal level in itself was not a sufficient condition for increasing the amplitude of the P300.

These data support "attention to the task" as an important factor concerning P300 amplitude. If one assumes that the incentive condition focussed the subjects attention on the task, then the waxing and waning of attention may be the major factor affecting increases or decreases in P300 amplitudes. That attention can wax and wane in an oddball task and not be reflected in the accuracy of counting or responding to targets is surprising. An attention interpretation fits well with the data presented in Figure 3 showing a recovery of P300 amplitude between blocks and a slow diminution in amplitude within a block of 35 targets. The gradual decrement between blocks is presumably due to the "waning of attention" across time. The repeat of instructions prior to each new block focussed attention on the task, though briefly, and resulted in higher amplitudes at the beginning of each block. Knowledge concerning the "end of session" and the incentive of money also focussed attention on the task. Thus, it appears that it is not the cognitive process indexed by the P300 that attenuates across blocks of testing, it is the failure of the task to invoke the process that results in attenuation of P300 amplitude.

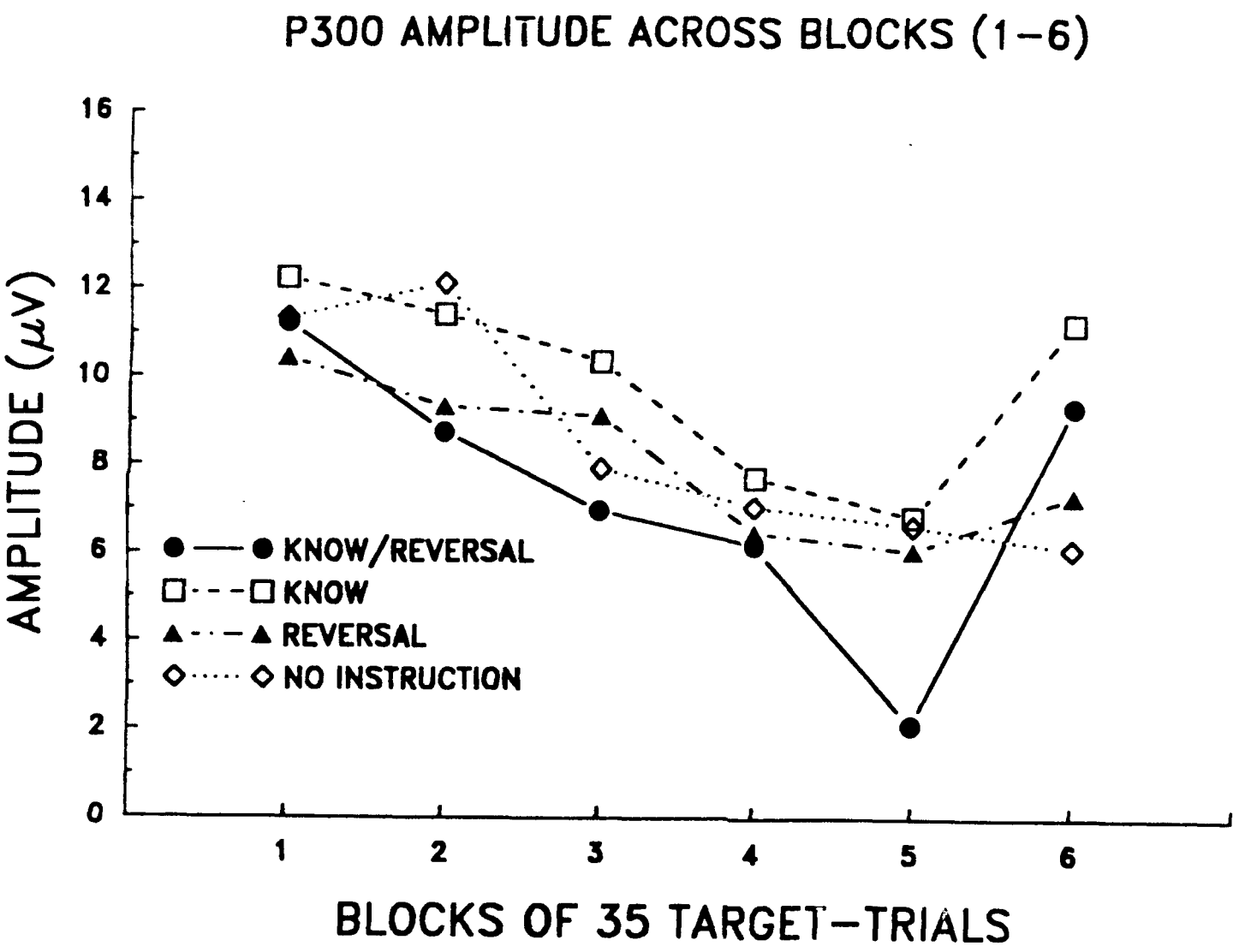


Figure 1. Mean P300 amplitudes across blocks for the four experimental conditions.

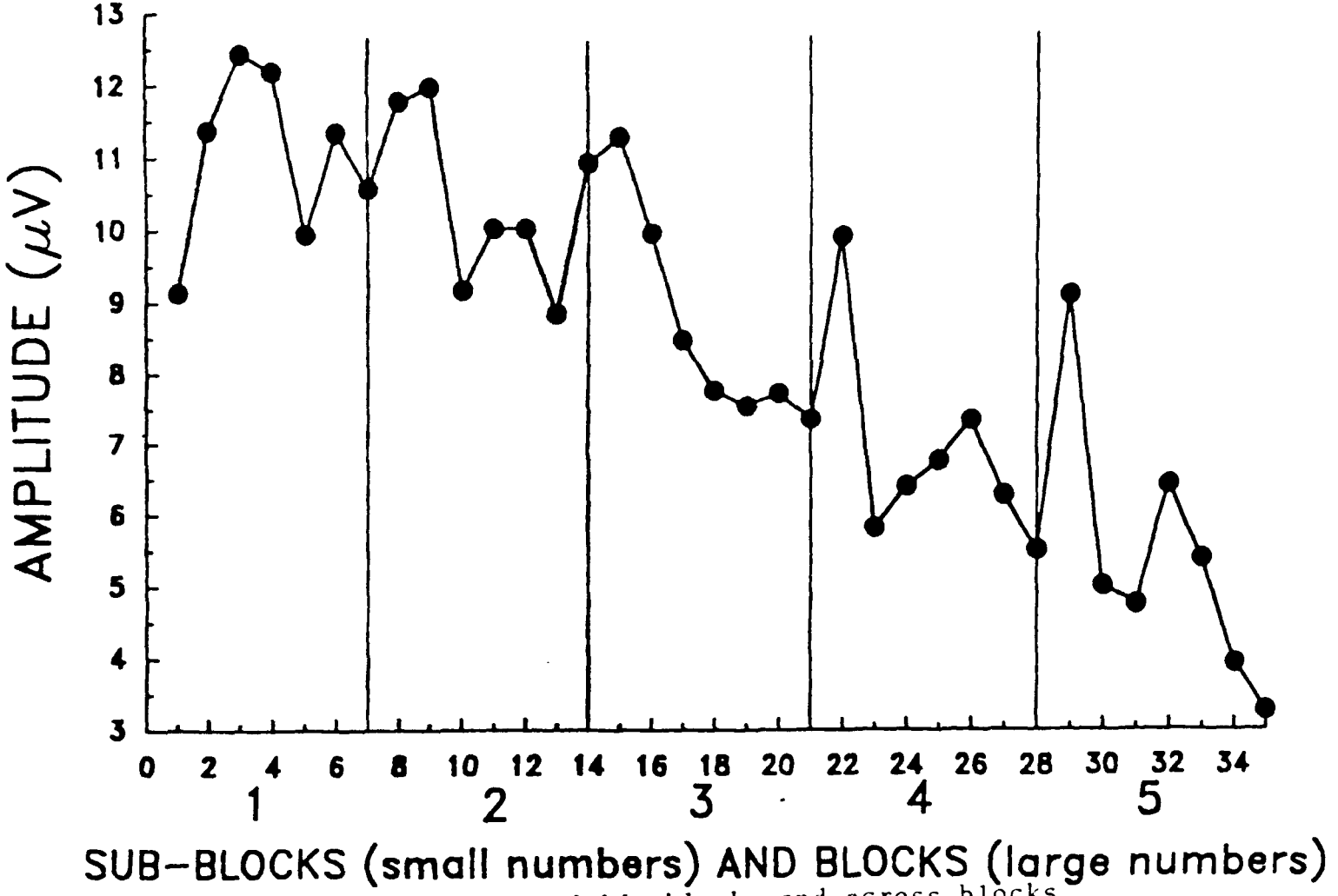
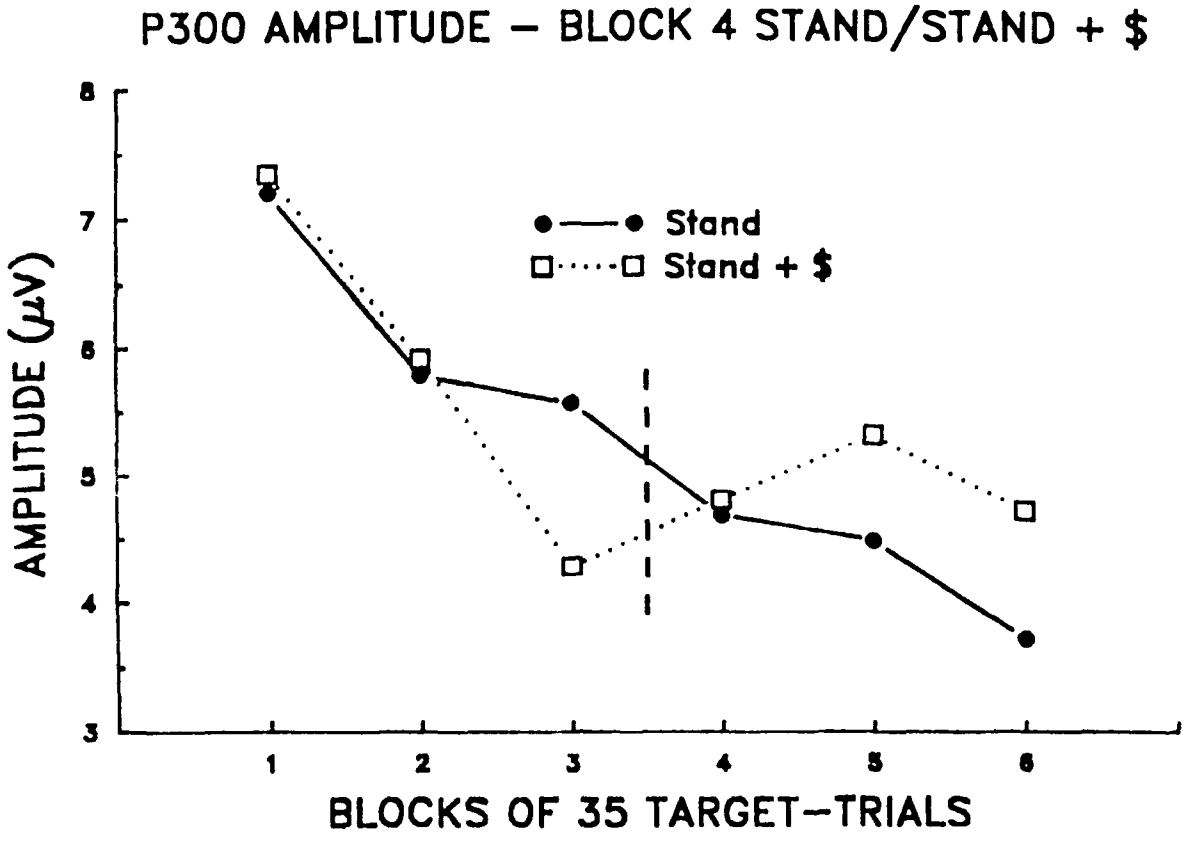


Figure 2. P300 amplitudes within blocks and across blocks. Each block of 35 targets is shown in sub-blocks of 5 targets.



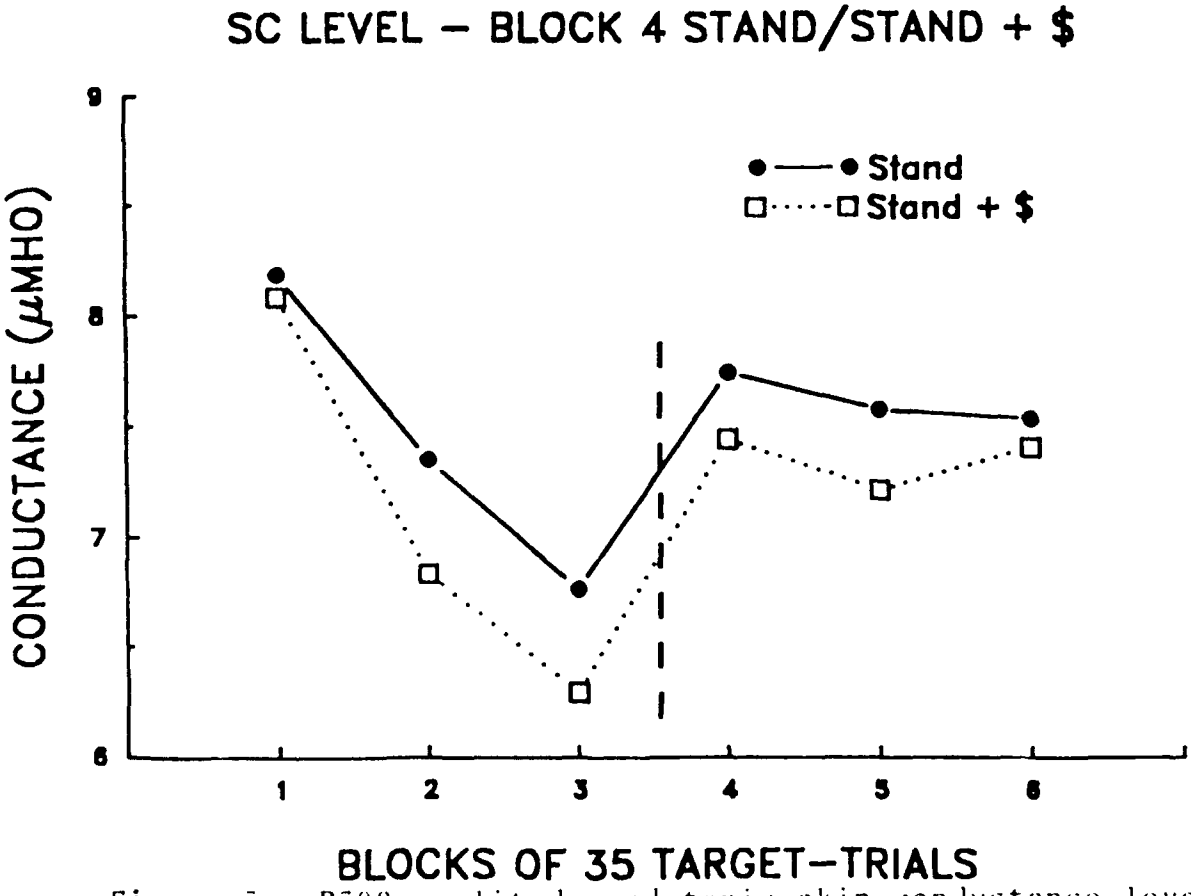


Figure 5. P300 amplitude and tonic skin conductance level prior to and following the treatment condition.

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6.2 Experiment 2 - ORIENTING RESPONSES AND EVENT-RELATED BRAIN POTENTIALS

Research on the orienting response (OR) has traditionally involved responses controlled by the peripheral autonomic nervous system. Thus, our understanding of OR mechanisms and their relationship to conditioning, learning, information processing, etc. is based on effector organ responses and the neural processes mediating those responses. Recently, efforts have been made to assess orienting in terms of central measures such as event related brain potentials. So called endogenous components of evoked brain potentials have properties in common with the orienting response. Importantly, both share the property of stimulus nonspecificity. That is, they are both evoked by stimulation of a variety of types and quality and both may occur when an expected stimulus is omitted.

Additionally, there are similarities in the eliciting conditions of orienting responses and the endogenous components of ERPs. Figures 1 and 2 illustrate an SCR, an event related brain potential, and the stimulus determinants of the two responses.

On the left side of Figure 1 is a schematic of a skin conductance response. This response is probably the most widely used and validated measure of the orienting response. Our focus here will be on SCR amplitude which is generally conductance at the peak minus conductance prior to the response. On the right side of Figure 1 is a list of the major stimulus factors related to SCR amplitude. Thus an SCR will vary in relation to stimulus novelty, i.e., the extent to which it deviates from expectations regarding its probability, modality, or timing; stimulus meaning, e.g., whether it is or is not a signal stimulus, or relevant or irrelevant to the subjects; and stimulus salience, for ex., its intensity.

On the left side of Figure 2 is a schematic of an event related potential. Event-related potentials -- ERPs, are measured from electrode placements at various locations on the scalp and several different components are typically identified. The focus here is on the positive going component at usually 300 or more ms post stimulus. The amplitude of this P300 component is generally measured in relation to a prestimulus baseline. On the right side of Figure 2 is a list of the major stimulus factors associated with P300 amplitude. Again, novelty, meaning, and salience are important. Thus, the eliciting conditions for the SCR and P300 are at least to a substantial degree, identical.

The fact that the SCR and the P300 have the same eliciting conditions does not necessarily mean that peripheral and central measures can be used interchangeably as indices of the orienting response. There are additional and fundamental properties of orienting responses that may or may not be shared by evoked potentials. For example, with repeated stimulation, orienting responses decline -- they are said to habituate. P300 in many studies, however, looks the same across many hundreds of stimulus

presentations -- there is no evidence of habituation. Another property associated with orienting responses is dishabituation, i.e., recovery of a habituated response following presentation of a test stimulus whose properties differ from the habituating stimulus. Dishabituation of ERPs has received little attention.

Unfortunately, comparisons of the properties of orienting responses and evoked potentials are severely limited by the differences in paradigms and analytic strategies used in their investigation. Figure 3 illustrates some of these differences.

One way of assessing whether ERP responses have the same properties as ORs requires study of ERPs using OR paradigms. Thus, ERPs need to be studied with longer ISIs, with nonsignal stimuli, and with procedures that provide for a finer-grained analysis of time effects. There are a handful of studies that have taken this approach.

Another way of assessing whether ERP responses have the same properties a ORs involves including traditional orienting response measures in studies using the ERP paradigm. This was the approach taken in the present experiment.

Method

An "oddball task" was used where subjects were presented target and nontarget tones (90 dB, 1.0 and 1.5 KH tones). The subjects were instructed to tap their foot when targets were detected and to keep count of the number of target stimuli presented. They were asked to ignore the nontarget stimuli. The stimuli were equiprobable. Both ERPs and SCR were recorded to 35 target and 35 nontarget stimuli. To assess dishabituation, a 4 KHz tone was presented between trials 30 and 31.

Results

Figure 3 presents the SCR averaged for seven blocks of five trials. As expected, amplitude decreased across the first six blocks. Although target amplitude was higher on block 1, there was no significant differences in overall level or rate of habituation for the target and nontarget stimuli. Unexpectedly there were no signs of dishabituation during the seventh trial block.

Figure 4 presents the SCR data across six blocks of two trials (data from the first 12 trials). Differences are apparent here on the very first block, but not on subsequent blocks.

With regard to SCR then, we found 1) habituation of the SCR response to both signal and nonsignal stimuli, 2) evidence of a larger initial (first two trials) response to the signal (target) stimulus, and 3) no sign of dishabituation.

Figure 5 presents the P300 data for seven blocks of five trials. The plotted data points at each block are a percentage of the first trial block for target and nontarget stimuli. Overall the amplitude of the target P300 was nonsignificantly larger than the nontarget P300. Data from the Fz lead only are presented. No significant differences were found at Cz and Pz.

It can be seen that the rate of habituation was greater for the target than the nontarget tone. The decrease for the nontarget tone was not statistically significant.

Dishabituation can also be seen. Relative to block six, the magnitude of the response was greater following the dishabituation stimulus for both tones (significantly greater for the target but not the nontarget).

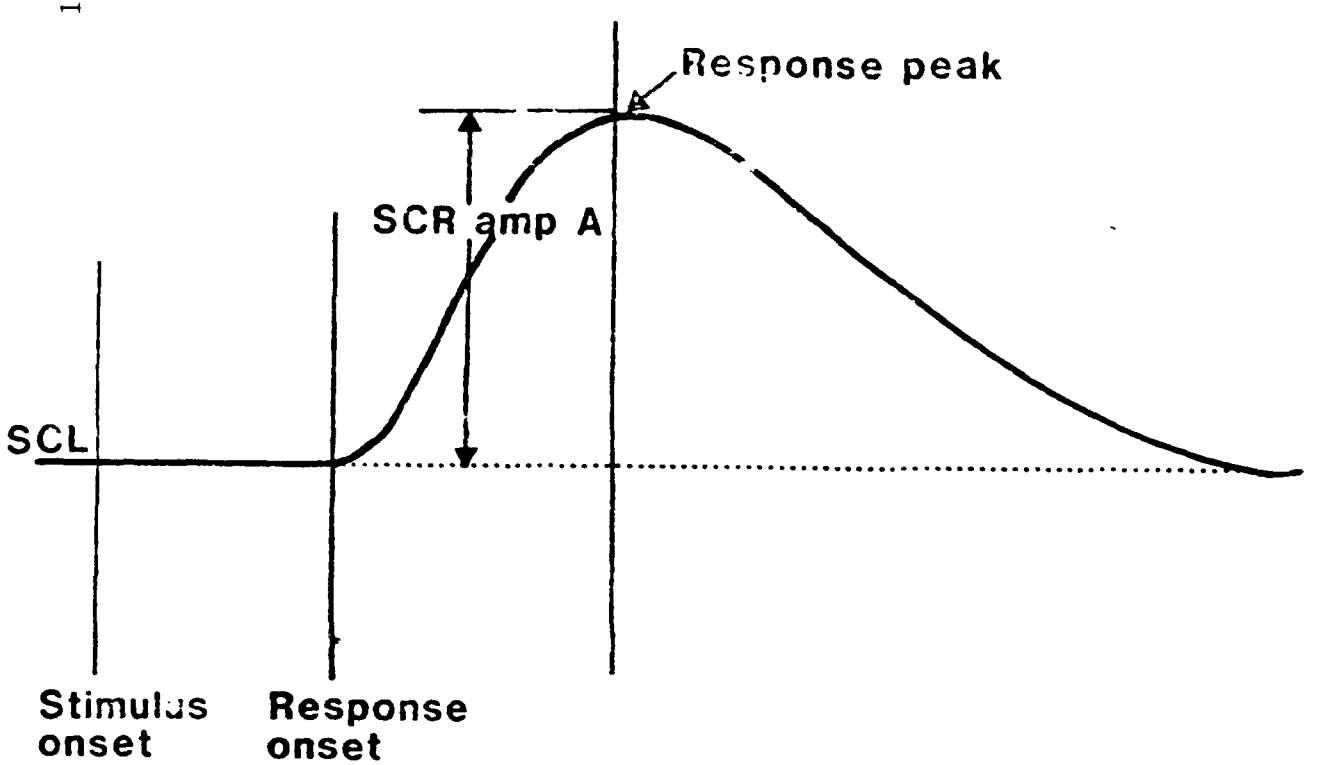
Figure 6 presents the ERP responses across six blocks of two trials (the first 12 trials). Again there is clear evidence of habituation with the target but not the nontarget stimulus. It appears that habituation rate was greater during the first two trial blocks.

Conclusions

In conclusion, the results of the present experiment can be summarized as follows. First we found habituation of both SCR and ERP responses to target stimuli in an "oddball" task. The habituation was rapid during the first 10-12 trials for both responses but decrements were evident across all 30 habituation trials. Interesting, while little or no differences were found in habituation of the SCR to target and nontarget stimuli, habituation of P300 amplitude was greater to the target stimulus. There was relatively little habituation of the P300 response to the nontarget. The differential habituation of SCR and ERP responses to target and nontarget stimuli may indicate fundamental differences in the characteristics of the underlying response systems. On the other hand, the ERP response may simply have been more sensitive to the experimental conditions. Further research is needed.

Second, we found evidence of dishabituation of the ERP response but not the SCR response. The latter was not expected. Again it may be either that OR and ERP response systems have different properties or that ERPs are more sensitive to the experimental conditions.

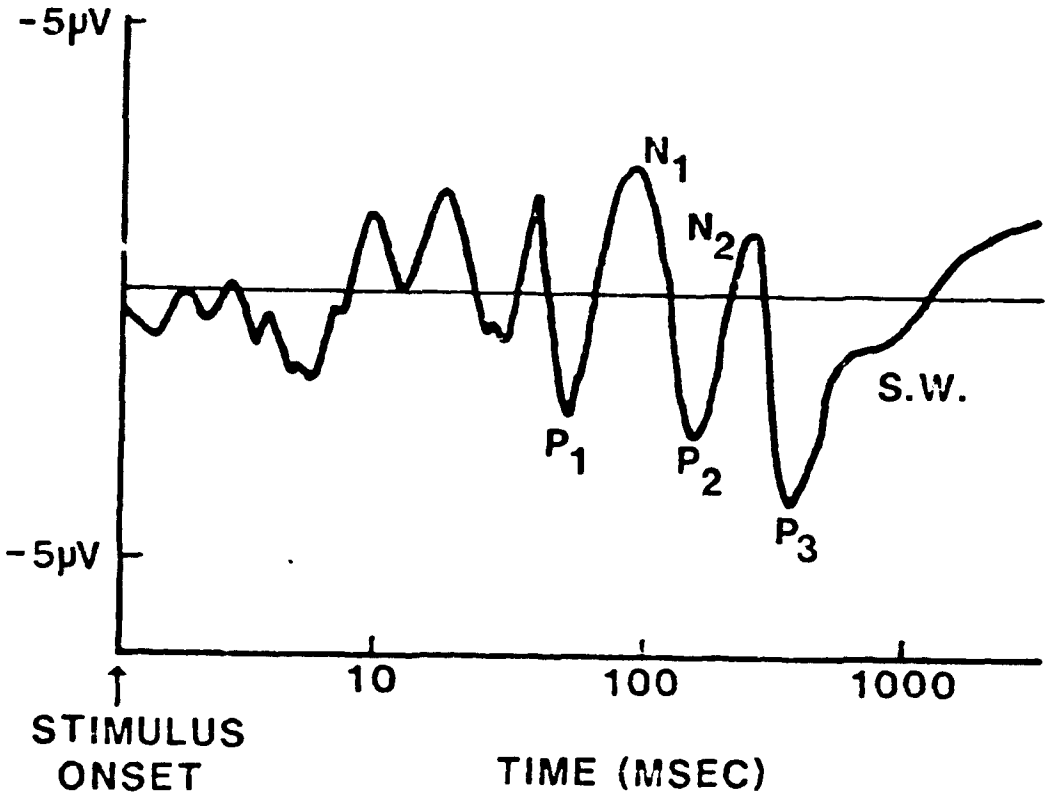
In conclusion, the present experiment suggests that including orienting response measures in ERP paradigms may be useful in providing information about the differences in the properties of ERP and OR response systems. It may be that the ERP is more sensitively related to the experimental conditions associated with orienting. Measures of central activity may provide the opportunity for a more direct analysis of the mechanisms producing the OR.



STIMULUS DETERMINANTS

Novelty
Meaning
Salience

Figure 1 - Illustration of a skin conductance response



STIMULUS DETERMINANTS

Novelty

Meaning

Salience

ERPs

Figure 2 - Illustration of an event-related potential

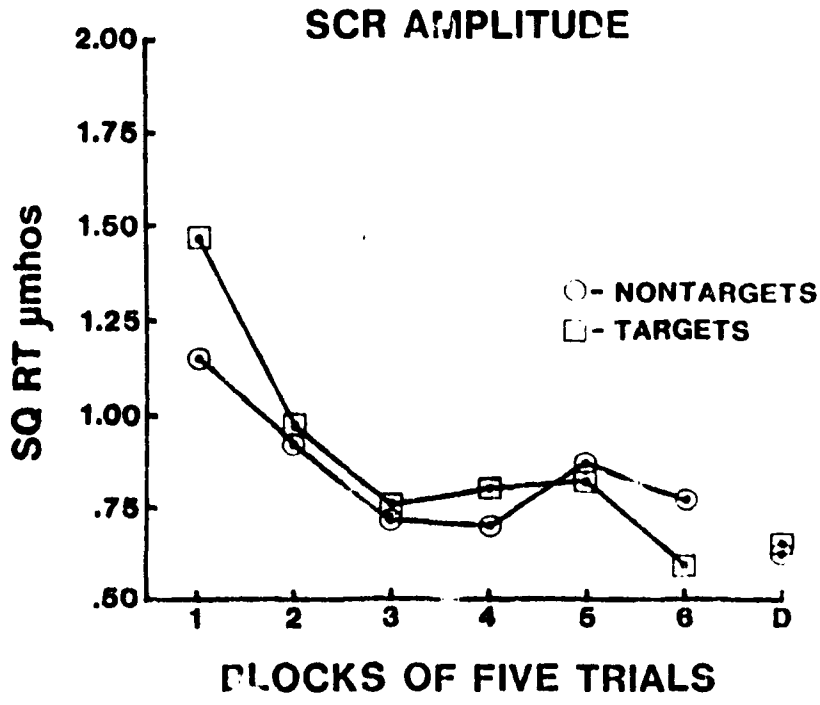


Figure 3 - SCR averaged for seven blocks of five trials

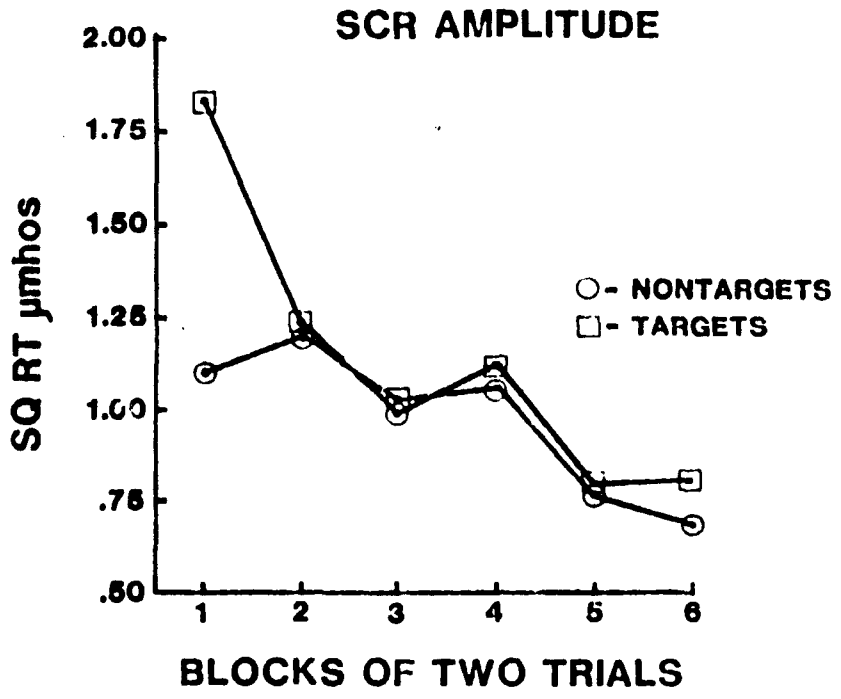


Figure 4 - SCR for six blocks of two trials

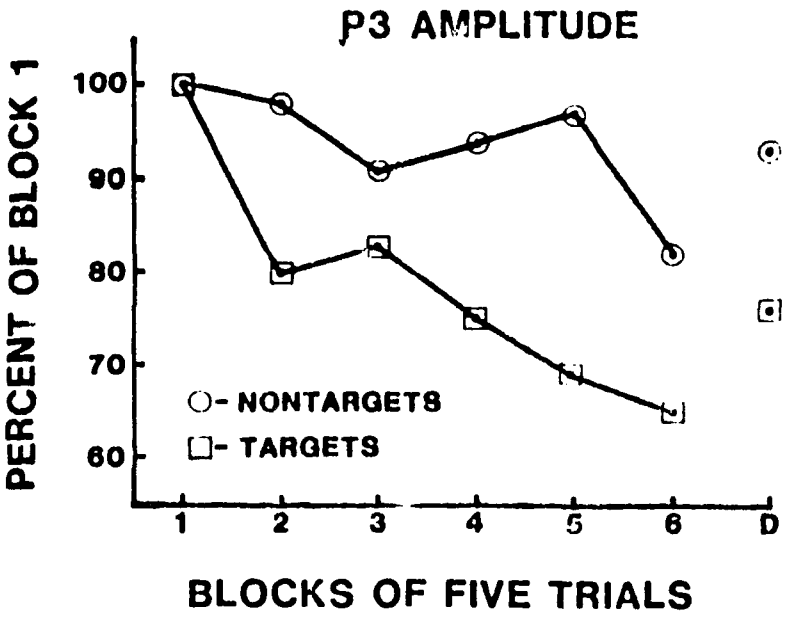


Figure 5 - P300 for seven blocks of five trials

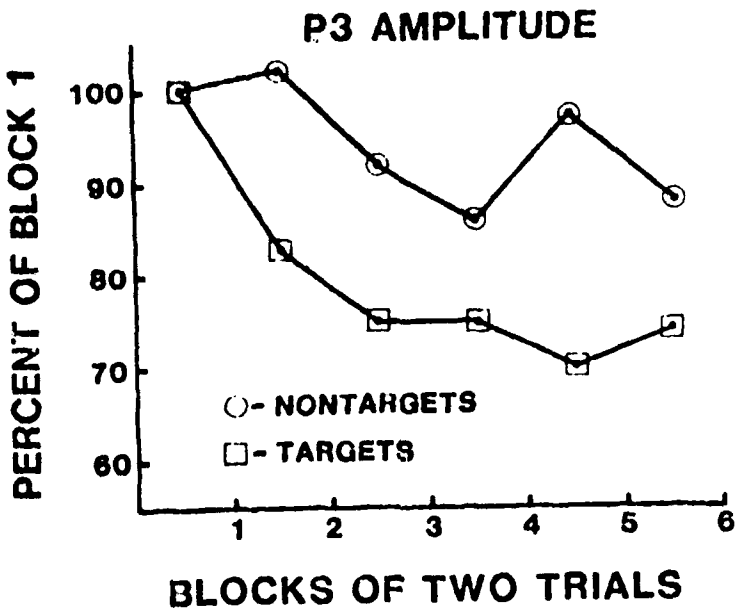


Figure 6 - P300 for six blocks of two trials

6.3 Experiment 3 - Event-related Potentials Across Three Phases of Pavlovian Conditioning

Pavlovian conditioning has received much systematic investigation since the original work by Pavlov in the early 1900's. As research has continued, a broader role has been assigned to Pavlovian conditioning as it relates to everyday behavior (Rescorla, 1988; Turkhan, 1989). In addition, more cognitive explanations have been offered to explain the conditioning process. The goal of the present paper is to provide a conditioning paradigm in humans in which brain event-related potentials (ERPs) can be used as indices of learning.

Much early research on human Pavlovian conditioning consisted of measuring conditioned eyeblink responses (e.g., Spence, 1956). Psychologists have more recently focused on psychophysiological measures to index Pavlovian conditioning in humans. These measures include muscle activity, skin conductance, heart rate, vasomotor activity, and event-related potentials. With the exception of event-related potentials, a substantial literature exists relating the above measures to Pavlovian conditioning. However, ERPs possess several advantages when compared with peripheral measures. First, researchers are optimistic that ERPs provide a more direct index of information processing than the peripheral measures. That is, factors associated with physiological mechanisms in the periphery are not an issue with ERPs. Second, ERPs occur concurrently with stages of information processing and return quickly to baseline. In contrast, peripheral responses generally exhibit a longer onset latency and a longer return to baseline. These characteristics of peripheral response systems restrict the experimental tasks which can be studied.

One traditional advantage of peripheral measurement has been the ability to perform a trial-by-trial analysis of conditioning. However, recent statistical procedures now enable the same type of single-trial analysis with various ERP waves (Birch, Lawrence, & Hare, 1988; Chase, McCarthy, Squires, & Schaneveldt, 1984; Fabiani, Gratton, Karis, & Donchin, 1987; Kenemans & Verbaten, 1988; Loveless, Simpson, & Naatanen, 1987; Pritchard, Brandt, & Barratt, 1986; Ruchkin & Glaser, 1976). The most common method of single trial ERP analysis involves digital filtering. This procedure reduces "noise" by filtering out those frequencies in the waveform that are not of interest.

As noted, the literature relating ERPs to Pavlovian conditioning is sparse. Of the ERPs, the CNV wave has received the most attention. Increases in CNV amplitude have been observed during eyeblink conditioning (Walter et al., 1964) and also during pairing of a tone with a phobic slide (Lumsden, Fenton, & Howard, 1980), a buzzer (Proulx & Picton, 1980), and a flash of light (Laffont, Bruneau, Jusseaume, & Lelord, 1977; Marineau, Garreau, Barthelemy, & Lelord, 1984). The CNV also

increases when subjects learn that one stimulus predicts a second stimulus which requires a speeded response (Low, Borda, Frost, & Kellaway, 1966) and when subjects learn paired associates (Peters, Billinger, & Knott, 1977). Furthermore, the CNV does not simply exhibit a linear increase in amplitude as one stimulus repeatedly predicts another stimulus. The above studies show an increase in the CNV during initial acquisition trials with a subsequent decrease thereafter. Findings relating the other ERPs to Pavlovian conditioning are few and mixed. For N100 amplitude, two studies report an increase as a result of conditioning (Begleiter & Platz, 1969; Syndulko et al., 1975) while several studies report no change (Hugdahl & Nordby, 1988; Marineau et al., 1984; Proulx & Picton, 1980). For P300, all but one study (Proulx & Picton, 1980) show an increase in amplitude during Pavlovian conditioning (Hugdahl & Nordby, 1988; Marineau et al., 1984; Peters et al., 1977; Raine & Venables, 1987; Syndulko et al., 1975). Only one study (Peters et al., 1977) examined changes in P300 across trials within the learning phase. In this study, P300 amplitude increased until the point where learning was complete and remained stable thereafter.

Few studies which assessed the relationship between Pavlovian conditioning and event-related potentials provided methodological details (several of the reports were abstracts). In addition, several studies used a methodology which rendered the findings ambiguous (Begleiter & Platz, 1969; Laffont et al., 1977; Marineau et al., 1984; Proulx & Picton, 1980). Finally, no study provided a detailed analysis of event-related potential changes across learning trials.

Although few studies have focused on brain measures of Pavlovian conditioning, the theoretical notions underlying several of these ERP components suggest that they are good candidates for indexing the learning process. For example, an increase in P300 amplitude has been related to increased stimulus significance, increased attentional resources to the stimulus and updating of memory. The CNV has been related to association, expectancy, attention and motivation. Many or all of the above processes would also seem to be involved when one stimulus becomes associated with (predictive of) another stimulus which is inherently significant, that is, during Pavlovian conditioning.

Research Hypotheses - Habituation Phase For the CNV to occur, a two-tone paradigm in which one stimulus predicts the occurrence of another stimulus is necessary. Therefore, since only single stimuli are presented during the habituation phase of testing, the CNV should be absent from all trials. The literature suggests that during the habituation phase the amplitude of N100 to both tones should be large initially and diminish rapidly to a stable amplitude (e.g., Ohman & Lader, 1977; Roth & Kopell, 1969). The P300 component should also occur initially but then decrease to near zero across the habituation phase (e.g., Courchesne, Courchesne, & Hillyard, 1978; Megela & Teyler, 1979).

Research Hypotheses - Acquisition Phase During

acquisition, the CS+ becomes predictive of an aversive stimulus (US) and the CS- becomes predictive of US absence. Based on previous research, CNV amplitude is predicted to increase during conditioning. Evidence also suggests that once the relationship is learned, the CNV will show a decrease in amplitude. These predictions fit the theoretical notion that the CNV reflects the cognitive resources involved in preparing to evaluate a stimulus. During learning, the subject will focus on whether the US follows each stimulus presentation. Therefore, a CNV may be observed initially to both CS+ and CS-. Further learning may result in an increase in CNV amplitude to CS+ and a decrease to CS-. However, if US absence following the CS- is interpreted by the subject as an event of "safety" then a CNV to CS- may also increase with further learning. Once learning is complete any CNV which has developed should decrease since no further evaluation of the presence or absence of the US is required.

Empirical findings and theoretical notions related to the N100 wave during acquisition are few and mixed. If N100 reflects attention to the auditory channel, one would expect a large N100 to both CS+ and CS- as long as aversive auditory events continue to be presented. That is, there should be an increase in N100 amplitude from the end of the habituation phase to the beginning of the acquisition phase.

The literature suggests that during the acquisition phase, the P300 to the CS+ should increase in amplitude as the subject learns the predictive relationship. The pattern of P300 amplitude once learning is complete is less clear. From a theoretical perspective, acquisition should involve memory associations being formed and the subject learning that the CS+ is a meaningful event. Both updating of memory and increased meaningfulness should result in an increase in P300 amplitude. Furthermore, the same rationale may apply to the CS-. That is, the CS- becomes predictive of a period of "comfort" (US absence). Learning this association may give meaningfulness to the CS-, thus resulting in an increased P300.

Research Hypotheses - Extinction Phase During the extinction phase the US is no longer presented. While CNV amplitude is expected to decrease toward the end of the acquisition phase, terminating US presentation will require the subject to reallocate resources to the events occurring at tone termination. Therefore, CNV amplitude to both tones may increase at the beginning of the extinction phase. As the extinction phase progresses, CNV amplitude should decrease. In addition, absence of the aversive auditory event should result in a decrease in attention to auditory input, and therefore, a decrease in N100 amplitude. Likewise, the meaningfulness of both CS+ and CS- should diminish, with a resulting decrease in P300 amplitude to both tones. Although both CS+ and CS- now predict "comfort", in relative terms comfort is not important since there are no longer periods of aversiveness.

Methods

Subjects Thirty undergraduate students (eighteen males,

twelve females) served as subjects (ages 18-24). Subjects received one hour of experimental credit for their participation.

Apparatus Event-related potentials and eye movements/blinks were recorded using Beckman silver-silver chloride electrodes. The EEG signals were amplified and filtered by Grass 7P122 D.C. amplifiers. For EEG signals, the time constant was 5.0 s and the high frequency filter was set to 35 Hz. The EOG (eye movements/blinks) was amplified by a Grass 7P1 D.C. Preamplifier (time constant = 5.0 s) and 7DA Driver Amplifier (high frequency filter = 35 Hz). The EEG and EOG were digitized at 200 Hz beginning 0.2 s prior to tone onset and continuing until 2.0 s after tone onset.

Procedure

Electrodes were affixed to the subject's scalp at Fz, Cz, Pz, A1, and A2 sites according to the 10-20 international system. Additional electrodes were placed above and below the right eye to record eye movements and blinks. An earclip electrode served as ground. Electrode impedance was kept below 5.0 Kohm for EEG sites and below 10.0 Kohm for EOG. Subjects were instructed that tones and loud noises would be presented. During the session, they were to simply listen to the stimulus presentations. They were also instructed to fixate their eyes on a small dot on the opposite wall.

Habituation phase Two tones (to be CS+ and CS-) of 1000 Hz and 2000 Hz were presented. Tone intensity was 75 dB and tone duration was 1.0 s. During this phase of testing, each tone was presented fifteen times. Order of tones was random (ISI=3-5 s).

Conditioning phase The conditioning phase followed the habituation phase without interruption (i.e., there was a 3-5 s interval). During this phase, one of the two tones (1000 Hz or 2000 Hz) was designated CS+ (to be paired with the US) and the other tone was designated CS-. The designation was counterbalanced across subjects. The interval between tones continued to be 3.0 to 5.0 s and each tone was presented fifteen times. Following each presentation of CS+, the US (white noise, 100 dB, 1.0 s) was presented. The US occurred concurrent with CS+ termination.

Extinction phase The extinction phase followed the conditioning phase without interruption. The parameters were identical to those of the habituation phase; each tone was presented fifteen times. The US was not presented during the extinction phase.

Immediately following the extinction phase, the subject completed a questionnaire which assessed the point at which they learned the CS+/US relationship.

ERP scoring Contribution of eye activity to scalp recordings was removed on each trial using a procedure described by Gratton, Coles, & Donchin (1983). After eye artifact was removed, waveforms were digitally filtered and CNV, N100 and P300 amplitude scored.

In order to facilitate the scoring of ERPs on a single trial

basis, waveforms were digitally filtered (BMDP1T) to eliminate frequencies outside the range of interest. Two different filtering procedures were used to improve the signal-to-noise ratio. The first procedure facilitated scoring of the CNV. With the stimulus parameters used in the present study, the CNV is a slow negative wave which begins from 260-460 ms after tone onset and increases in amplitude until occurrence of the second stimulus 1000 ms after tone onset (Tecce, 1972). This pattern of amplitude change implies that much of the power of the CNV consists of frequencies below 1.0 Hz. Therefore, a filter was used which allowed the slower frequencies (below 2.0 Hz) to pass but attenuated higher frequencies. The CNV was scored as the mean amplitude of the waveform from 900-1000 ms post-tone onset (100 ms prior to US onset) minus the amplitude of the 200 ms baseline prior to tone onset.

The second filtering procedure was designed to facilitate scoring of N100 and P300 peaks. The power of these latter waves resides in frequencies of 3.0 Hz (P300) to 6.0 Hz (N100) (Pritchard, Brandt, & Barratt, 1986; Ruchkin & Glaser, 1976). Therefore, the second filter (which actually consisted of two filters) attenuated frequencies above and below the 3.0 to 6.0 Hz range. Amplitude scores for the waveform peaks were defined as the maximal peak within a specified latency range minus the mean amplitude of the baseline. The latency ranges following onset of CS+ and CS- were 80-150 ms for N100 and 290-450 ms for P300. These latency ranges were determined by inspecting the average waveforms. If no peak was found within a given latency range, the amplitude and latency values were interpolated based on the scores of the two trials preceding and two trials subsequent to the missing trial.

Results

Amplitude scores were recorded for each tone presentation across each phase of the study (total presentations = 45 CS+, 45 CS-). Examination of the data revealed substantial changes in component amplitude and latency from one trial to the next trial within a test phase, i.e., considerable variation from trial to trial. Therefore, the scores were averaged across every three trials to create five blocks of three trials for each phase of the study.

The post-session questionnaire revealed that eleven of the thirty subjects could not report the relationship between the CS+ and the US during the acquisition phase. Therefore, a Group factor (learners, non-learners) was added to the analyses.

Initial analyses revealed that the scalp site interacted rarely with the other factors. Therefore, since amplitudes were generally largest at the Cz site, analyses reported below utilized scores from the Cz site only and incorporated the Group (learners, non-learners) factor. Thus, for each phase of testing, a three-way mixed ANOVA was performed, with the between factor being Group (learners, non-learners) and the within factors being Tone type (CS+, CS-) and Block (Blocks 1-5). (Note: Greenhouse-Geisser degrees of freedom adjustment was used

for repeated measures analyses and corresponding p values reported.)

While most predictions in the present study related to ERP changes across a particular phase of testing, several predictions related to changes occurring from the end of one phase to the beginning of the next phase. To test the latter predictions, a three-way mixed ANOVA was performed, with the between factor being Group (learners, non-learners) and the within factors being Tone type (CS+, CS-) and Block (Block 5 to Block 1).

Habituation Phase - CNV wave While it was predicted that no CNV would be observed during the habituation phase (since no second stimulus followed the tones), results showed that a small CNV was present. The pattern of CNV amplitude for learners and non-learners across phases is illustrated in Figure 1. For both groups, a small CNV was present on Block 1 for both tones. The CNV then asymptoted to a level near 2.0 uV across Blocks 2-5. The three-way ANOVA (Group X Tone type X Block) revealed no significant effects for CNV amplitude across the habituation phase.

N100 peak. Figure 2 shows the amplitude of N100 across the three test phases for learners and nonlearners. Focusing first on the N100 peak for learners, it can be seen that N100 amplitude to both tones decreased as predicted from 14.7 uV to 11.5 uV across the habituation phase. Furthermore, it appeared that by the end of the habituation phase N100 amplitude still did not reach an asymptotic level. The pattern of N100 change appeared more variable for the non-learners. Although there appeared to be a decrease for learners, the three-way analysis revealed no significant effects for N100 amplitude across the habituation phase.

P300 peak. As predicted, P300 to both tones decreased rapidly to an asymptotic level across the habituation phase (see Figure 3). The pattern of change was similar for both learners and non-learners. Across conditions, P300 amplitude decreased from 9.7 uV on Block 1 to 7.0 uV on Block 2. The amplitude stabilized near 7.0 uV across Blocks 2-5. The three-way analysis showed only a main effect for Block ($F(4,112)=5.42$, $p.<.01$), which indicated that the initial decrease in both groups was significant.

Acquisition Phase - CNV wave. For learners and non-learners the CNV to both tones showed a marked increase in amplitude across the acquisition phase (see Figure 1). This increase continued through the end of the 15 acquisition trials with no indication that the CNV measure had asymptoted. Furthermore, there appeared to be some differences between the CS+ tone and the CS- tone and for learners and non-learners. The increase in CNV for both groups appeared more marked for the CS+ than for the CS-. The CNV to the CS+ increased from about 2.0 uV to about 13.0 uV across the acquisition phase, while the CNV to CS- only increased from about 4.0 uV to about 8.5 uV. It can also be seen in Figure 1 that the nature of the CNV increase in learners appeared different from that of non-learners. For learners, the

CNV increased markedly across Blocks 1-3, with the CNV to CS+ becoming larger than the CNV to CS- on Block 2. For non-learners, the CNV did not show a marked increase until Blocks 3-5, and the CNV to CS+ did not become larger than the CNV to CS- until Block 4. The reliability of the above observations was assessed with a three-way ANOVA (Group X Tone type X Block). The analysis confirmed that the main effect for Block was significant ($F(4,112)=5.23$, $p.<.01$), but that the interactions of Tone type X Block and Group X Block described above did not reach significance. As noted above, CNV changes were still occurring at the end of the acquisition phase. The differential effect for CS+ and CS- (Tone type X Block interaction) may have been more apparent if additional acquisition trials had been given.

N100 peak. It was predicted that the amplitude of N100 to both tones would increase from the end of the habituation phase to the beginning of the acquisition phase. Figure 2 shows that this increase was apparent for learners but not for non-learners. For learners, the amplitude of N100 to both CS+ and CS- increased 2.5 uV from Block 5 of the habituation phase to Block 1 of the acquisition phase. For non-learners, there was no systematic change across this same period. A three-way ANOVA (Group X Tone type X Block) assessed changes from Block 5 of habituation to Block 1 of acquisition. This analysis showed no significant effects, including the Group X Block interaction described above ($p=.09$). However, the considerable variability in the non-learners group may have masked the differences in the learners group. Therefore, to eliminate variability due to non-learners and to focus on N100 changes for the learners, a two-way within ANOVA (Tone type X Block) was performed for the learners group only. This analysis did show a main effect for Block ($F(1,18)=4.76$, $p.<.05$), thus providing evidence for the increase in N100 amplitude from the end of habituation to the beginning of acquisition (for learners).

The amplitude of N100 across the acquisition phase showed unexpected but interesting results. For both learners and non-learners, amplitude of N100 to CS+ was generally larger than to CS- across the acquisition phase. From the figures it appeared that N100 amplitudes to both CS+ and CS- were similar on Block 1. Following Block 1, N100 amplitude to CS+ increased to a maximum value of 16.7 uV on Block 3 (non-learners) or Block 4 (learners) and showed a slight decrease across the remaining trials. The three-way analysis revealed only a main effect for Tone type, $F(1,28)=6.09$, $p.<.05$. The Tone type X Block interaction was not significant.

P300 peak. Contrary to expectations, the amplitude of P300 to CS+ did not show an increase across the acquisition phase. Figure 3 suggests that, for learners only, the amplitude of P300 to CS- showed an increase across the acquisition phase. For learners, the P300 to CS+ decreased from Block 1 (5.5 uV) to Block 4 (3.5 uV), with an increase from Block 4 to Block 5 (5.0 uV). Further, it appeared that the amplitude of P300 to CS- was still increasing at the end of the acquisition phase. In

contrast to the P300 effects observed for learners, Figure 3 shows that, for non-learners, the amplitude of P300 to both the CS+ and the CS- across the acquisition phase was variable, with a general decrease in amplitude of 2.0 uV for both tones.

While the above observations suggested that learners showed an increase in amplitude of the P300 to CS-, the three-way interaction (Group X Tone type X Block) was not significant. In addition, no other effects were significant. Although the three-way interaction did not reach significance, the trial-to-trial variability in the non-learner group may have masked the CS+/CS- differences in the learner group. To eliminate the variance contributed by non-learners and to focus on changes in P300 amplitude for learners, a two-way within ANOVA (Tone type X Block) was performed for the learners group only. The two-way analysis did show a main effect for Tone type, $F(1,18)=4.83$, $p.<.05$, but did not reveal a Tone type X Block interaction. Thus there was some statistical evidence for a larger P300 to CS- than to CS+ in the learners group. As noted above, P300 amplitude was still changing at the end of the acquisition phase. Therefore, additional acquisition trials may clarify these interesting differences.

Extinction Phase - CNV wave. During the acquisition phase (see above), the CNV increased across blocks. As expected, during the extinction phase the CNV decreased from Block 1 to Block 5 (see Figure 1). Like the acquisition phase, the effect appeared delayed in the non-learner group. The three-way ANOVA confirmed these observations. There was a main effect for Block, $F(4,112)=4.15$, $p.<.01$, and a Group X Block interaction, $F(4,112)=3.27$, $p.<.05$.

N100 peak. As described above, the acquisition phase resulted in a larger N100 to CS+ than to CS-. However, the extinction phase resulted in similar amplitudes for both CS+ and CS-, with amplitude to both tones showing an overall expected decrease across extinction blocks. Although an overall decrease was observed for both learners and non-learners, learners appeared to show an initial increase in N100 amplitude from Block 1 to Block 2 of the extinction phase which was not apparent for non-learners. The overall decrease was reflected by a main effect for Block, $F(4,108)=3.84$, $p.<.01$. No other effects were significant.

P300 peak. It can be seen in Figure 3 that P300 amplitude, in general, increased from Block 5 of the acquisition phase to Block 1 of the extinction phase. The three-way ANOVA (Group X Tone type X Block) which assessed these changes from Block 5 to Block 1 showed a main effect for Block, $F(1,28)=5.05$, $p.<.05$. No other significant effects from the end of acquisition to the beginning of extinction were observed.

Following this initial increase in P300 amplitude at the beginning of the extinction phase, there appeared to be a small (2.0 uV) decrease in the amplitude of P300 to CS+ across the extinction phase. The amplitude of P300 to CS- changed little across these same periods. The three-way analysis which assessed

changes across the extinction phase showed no significant effects for P300 amplitude.

Discussion

A summary of the results across the habituation, acquisition, and extinction phases of testing reveals several systematic changes. The habituation phase was designed to obtain stable ERP responding prior to beginning the acquisition phase. With the possible exception of N100 amplitude, the habituation phase served its purpose. During the acquisition phase, the CNV, N100, and P300 peaks appeared to index aspects of conditioning. For the CNV, amplitude to CS+ and CS- increased across the acquisition phase. Furthermore, the CNV increase appeared more marked for CS+ than CS-, and the CNV increase seemed to occur later for non-learners. For N100, the amplitude to CS+ was larger than to CS- for both learners and non-learners. For P300, there was some evidence in the learners group that P300 amplitude was larger to CS- than to CS+ across the acquisition phase. During the extinction phase the amplitude of CNV and N100 decreased. Like acquisition, the CNV decrease occurred later in the extinction phase for the non-learners.

Habituation Phase

The focus of the present study was on ERP changes as one learns predictive relationships during the acquisition phase. However, the habituation phase serves an important role. The habituation phase is designed to provide a stable baseline of ERP amplitude prior to presenting acquisition trials. There is a substantial literature which shows that initial presentations of novel stimuli (tones) result in relatively large orienting responses. When the stimuli are irrelevant (i.e., no task required), the orienting response decreases to a stable level within a few trials. In the present study, this stable level was generally achieved.

CNV wave. It was hypothesized that the CNV would be absent during the habituation phase since a second stimulus never followed the tones. However, there appeared to be a negative component on Block 1 (6.0 uV), with a slight negativity persisting across Blocks 2-5 (2.0 uV). This wave may be the "sustained potential" described by Picton, Woods, and Proulx (1978) which occurs in response to the continuation of a stimulus. Naatanen and Picton (1987) have also described a "processing negativity" which is observed as long as an attended stimulus is processed.

N100 peak. A number of studies demonstrate habituation of N100 amplitude to "nontarget" tones (e.g. Ohman & Lader, 1977; Roth & Kopell, 1969; Woods & Elmasian, 1986). The present results showed a trend in this direction. Failure to fully replicate these findings and to confirm predictions may relate to two factors. First, instructions to subjects to " ... listen carefully to the sounds that you hear" may have given the tones some "target" value. In addition, additional habituation trials would have likely resulted in a statistically significant decrement in N100 amplitude.

P300 peak. As hypothesized, P300 amplitude decreased across the habituation phase. This finding is consistent with many other studies showing rapid habituation of the P300 to irrelevant stimuli (Courchesne, Courchesne, & Hillyard, 1978; Fruhstorfer, 1971; Lutzenberger, Schandry, & Birbaumer, 1979; Megela & Teyler, 1979; Picton & Hillyard, 1974; Squires et al., 1973). However, in the present study P300 amplitude did not approach zero. Rather, P300 amplitude stabilized with an amplitude of 7 uV over the last three blocks of the habituation phase. Like the interpretation of the N100 peak, this latter effect may have resulted from the instructions given to the subjects. Also, the mean interstimulus interval between presentations of the same tone was ten seconds. This is longer than most studies which assessed P300 habituation to irrelevant stimuli.

Acquisition Phase

The acquisition phase was the primary focus of the present study. Interest was in changes in central ERP measures as a result of making one of two tones predictive of an aversive event. From a traditional perspective, strict evidence for conditioning requires a change in response to the CS+ stimulus which is not observed for the CS- stimulus. However, more recent views of conditioning stress the relationships established between all events in the environment, not simply the relationship between the CS+ and the US. In the following sections, the results of the acquisition and extinction phases are evaluated in light of both traditional definitions of conditioning and more recent emphasis on the cognitive constructs involved in the subject's interpretation of the stimuli.

CNV wave. In the present study, the CNV to both CS+ and CS- increased markedly across the acquisition phase. While an initial increase in CNV to both tones was predicted, it was further hypothesized that the CNV to CS+ would increase until learning was complete and then the CNV to CS+ would decrease. However, the fact that one-third of the subjects could not report the CS+/US relationship suggests that additional acquisition trials may be required for complete learning.

After the initial CNV to CS-, it was predicted that the CNV to CS- may also increase (and subsequently decrease) if the absence of the US became an event of "comfort". Of the studies which examined the CNV component during conditioning, no study reported whether there was a CNV to the CS- stimulus. In fact, a number of the studies did not incorporate a CS- into the paradigm (Laffont et al., 1977; Marineau et al., 1984; Walter et al., 1964). Traditional views of Pavlovian conditioning emphasize the absence of a response to the CS- stimulus. However, in order to learn a predictive relationship one must learn a discrimination. That is, not only must an individual focus on the event following the CS+, the individual must prepare to evaluate the absence of that event following the CS-. The appearance of the CNV component following the CS- may reflect this latter preparation.

In addition to the above effects, the increase in CNV amplitude to both tones appeared delayed for non-learners

relative to learners. This is particularly interesting since non-learners exhibited a marked increase in CNV amplitude to CS+ across the last acquisition block. This increase resulted in a CNV amplitude to CS+ on Block 5 that was actually larger than the CNV on Block 5 for learners. In fact, several of the subjects classified as "non-learners" made statements like "It seemed that the noise followed one of the tones more often than the other, but I'm not sure". These findings suggest that changes in the CNV may predict awareness of relationships in the environment.

N100 peak. The amplitude of N100 proved to be a measure which indexed a discrimination between CS+ and CS- during the acquisition phase for both learners and non-learners. Two general factors which affect N100 amplitude include arousal level and attention to stimulation (Naatanen & Picton, 1987). Therefore, it was predicted that the aversive events occurring during the acquisition phase would increase both of these factors, and that N100 to both CS+ and CS- would be increased during the acquisition phase relative to the habituation phase. The results supported this prediction for learners, but also revealed a differential increase in amplitude to the CS+ relative to the CS- for both learners and non-learners.

Although several studies suggest an increased N100 when a particular channel of stimulus input is attended (e.g., Hillyard, Hink, Schwent, & Picton, 1973; Schwent & Hillyard, 1975), little research has focused on the effects when a particular stimulus within a channel of input is attended. The present results suggest that N100 amplitude may index attention to a particular stimulus and may be a central measure of Pavlovian conditioning. That is, Pavlovian conditioning may result in increased attention to the CS+ stimulus relative to the CS- stimulus.

It is interesting that the above findings and discussion for N100 apply to both learners and non-learners. In fact, the maximum amplitude to the CS+ tone during acquisition was slightly larger for non-learners and occurred one block earlier for them. One can speculate that these findings reflect some form of conditioning (learning) to which the subject remains verbally unaware. This type of learning may relate to why so many phobic individuals cannot verbalize the event(s) which resulted in their fearful associations.

P300 peak. In addition to N100 amplitude showing a discrimination between CS+ and CS- during acquisition, there was some evidence that P300 amplitude also showed this discrimination. Furthermore, this discrimination was only observed for learners. However, the pattern of P300 change for learners was contradictory to what was hypothesized. There appeared to be an initial suppression of P300 amplitude to both the CS+ and CS- at the beginning of the acquisition phase. While P300 amplitude to the CS- recovered to a baseline of 7.0 to 8.0 uV (level at the end of the habituation phase), P300 amplitude to CS+ remained small (4.0 to 5.0 uV) throughout the acquisition phase. These results are difficult to interpret since no current theory of the P300 can incorporate these findings. Learning that

CS+ predicted the US and that CS- predicted the absence of the US was expected to make both tones more "meaningful". In addition, the memory updating process involved in this learning was expected to increase P300 amplitude, especially to the CS+. Furthermore, the present results with P300 are contradictory to one previous report of a larger P300 to the CS+ than the CS- during acquisition (Hugdahl & Nordby, 1988). One can speculate regarding this unexpected outcome.

The present study may be different from other studies which examined factors affecting P300 amplitude. The aversive stimuli presented during the acquisition phase may have resulted in a state of fear or extreme arousal due to the aversive event (intense noise). While both the present study and other studies (e.g., Roth, Blowers, Doyle, & Kopell, 1982) have recorded large P300s to intense auditory stimuli, P300s in the presence of stimuli predicting aversive events have not been examined. Perhaps the stage of information processing indexed by the P300 is adversely affected under these conditions. In support of this notion, there is a body of evidence suggesting that performance on some tasks is impaired under high levels of emotional arousal (e.g., Easterbrook, 1959; McKenna, 1986; Morris, Davis, & Hutchings, 1981; Sarason, 1980; Wine, 1980).

The above interpretation must be explained in relation to the findings of Hugdahl and Nordby (1988). These researchers found a larger P300 to CS+ than to CS- as a result of eight acquisition trials. The stimuli used by Hugdahl and Nordby (1988) were similar to those in the present study. However, in their study the mean time interval between presentations of the white noise was eighty seconds, whereas the mean interval in the present study was eight seconds. Therefore, the level of arousal at the onset of each tone may have been much lower in their study. While this interpretation may be incorrect, other interpretations of the P300 findings are difficult to formulate. It is clear that current theoretical interpretations of the P300 must be modified to explain the current findings.

Extinction Phase The extinction phase is used in Pavlovian conditioning studies to observe a reduction in conditioned responding once the unconditioned stimulus is removed from the environment. In the present study, this reduction was observed for both CNV and N100 amplitude.

CNV wave. A decrease in CNV amplitude to both the CS+ and the CS- was observed across extinction trials, with the decrease occurring later for the non-learners. It was predicted that the CNV would decrease across extinction trials as the subject learned that no second stimulus followed tone presentation. This finding supports the notion that the CNV reflects the cognitive resources involved in preparing to evaluate an upcoming stimulus event. As the subject learned that a second stimulus was no longer presented, there was no need for cognitive preparation. The difference observed between learners and non-learners during the extinction phase fit well with the differences observed during the acquisition phase. During acquisition, the CNV

results suggested that it took longer for non-learners to begin to learn the CS+/US association. Likewise, during extinction, the CNV results suggest that it took longer for non-learners to realize that no further aversive stimuli would be presented.

N100 peak. It was hypothesized that elimination of the aversive stimulus would lead to a decrease in attention to the tones, as indexed by N100 amplitude. This prediction was confirmed. While there was an overall decrease in N100 amplitude across the extinction phase, learners appeared to show an initial increase in N100 amplitude from Block 1 to Block 2 before showing a subsequent decrease. Non-learners did not exhibit this initial increase. One can speculate that once learners realized that the sequence of stimulus events had changed, learners reoriented to the tones in order to reevaluate the stimulus environment. Non-learners may not have made this effort.

P300 peak. No significant change in the P300 component was observed across extinction trials, although learners showed a slight decrease. While a decrease in amplitude was predicted, the prediction was based on the assumption that "meaningfulness" and memory updating would be relevant factors during the acquisition phase. As discussed above, this assumption was contradicted by the findings during the acquisition phase. However, one aspect of the extinction phase is interesting and may support the "fear/arousal" interpretation provided for the P300 acquisition data. While P300 amplitude to CS- became larger across acquisition trials than P300 amplitude to CS+ (learners only), this difference was no longer apparent at the beginning of the extinction phase (and throughout the extinction phase). That is, from the end of the acquisition phase to the beginning of the extinction phase, the amplitude of P300 to CS+ recovered to baseline levels. Therefore, the association of the CS+ with fear/arousal may have extinguished rapidly once presentation of the US was terminated.

Summary

The present study showed that a discriminative Pavlovian conditioning paradigm resulted in systematic changes in the CNV, N100, and P300 event-related potential measures. During the acquisition phase of testing, CNV amplitude increased to both the CS+ and CS- tones. This may reflect the subject's preparation to evaluate both the presence of the US following CS+ and the absence of the US following CS-. In addition, the above effects seemed to occur later in the acquisition phase for subjects who could not report the CS+/US association (non-learners). Discriminative responding during the acquisition phase was evidenced by a larger N100 to CS+ than to CS- (for both learners and non-learners) and a smaller P300 to CS+ than to CS- (for learners only). The N100 results may reflect increased attention to the CS+ stimulus relative to the CS- stimulus during the acquisition phase. The P300 results may reflect an association with extreme arousal which impairs the processing indexed by the P300. Both CNV amplitude and N100 amplitude showed extinction across the final phase of testing. Overall, the results suggest

that the use of central measures in addition to peripheral measures, may provide a more complete understanding of the learning process underlying Pavlovian conditioning.

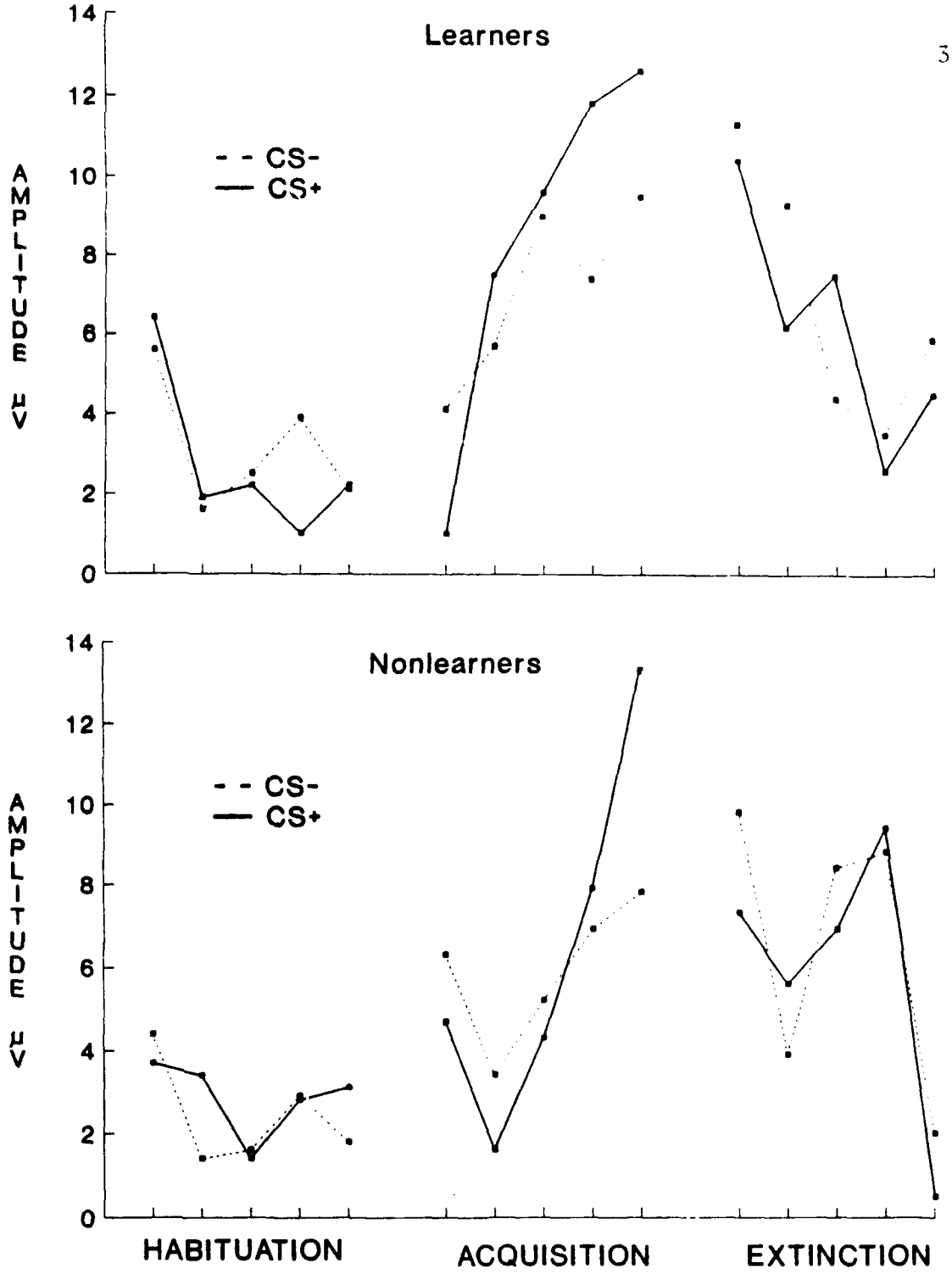


Figure 1 - CNV amplitude for learners and nonlearners across the three test phases

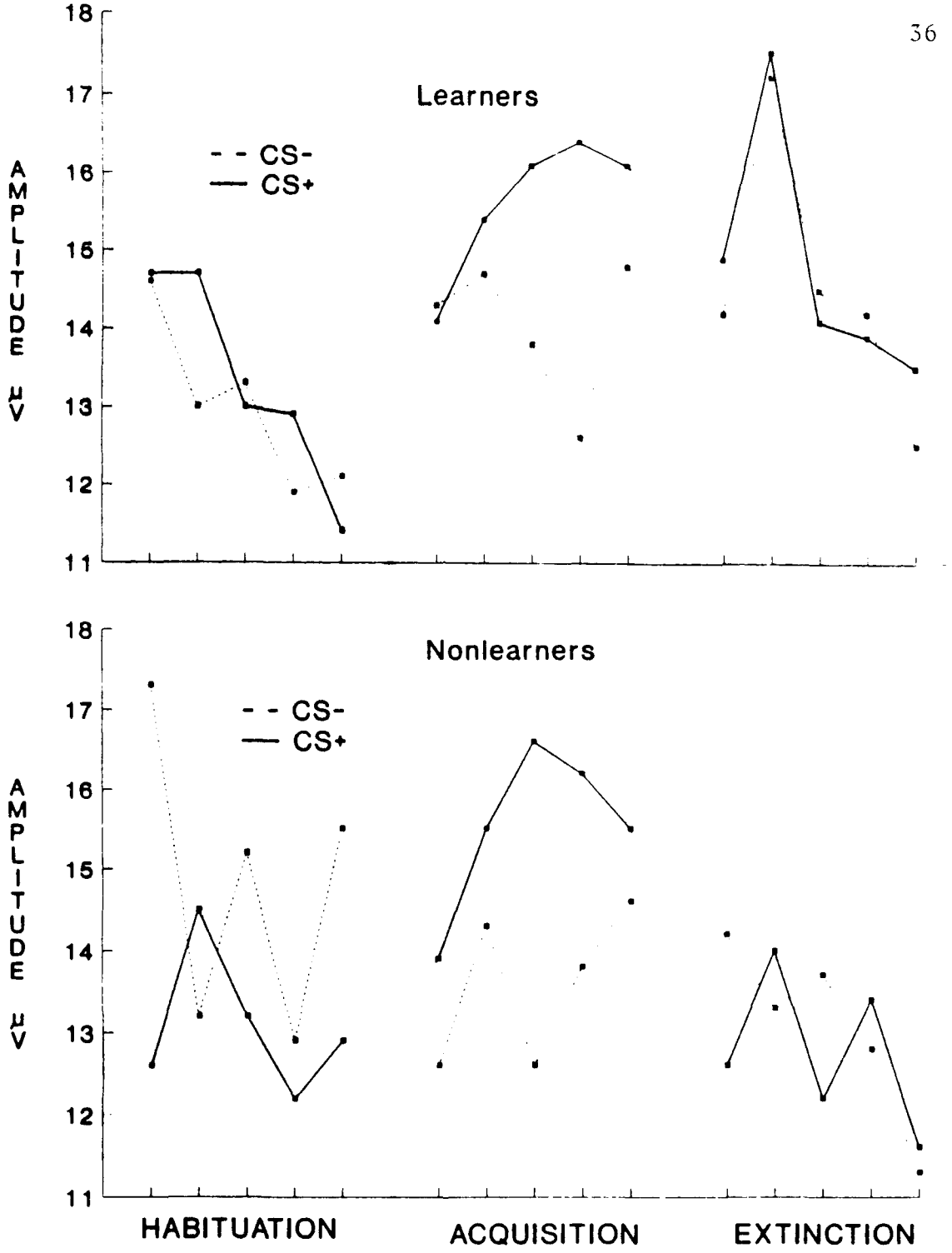


Figure 2 - N100 amplitude for learners and nonlearners across the three test phases

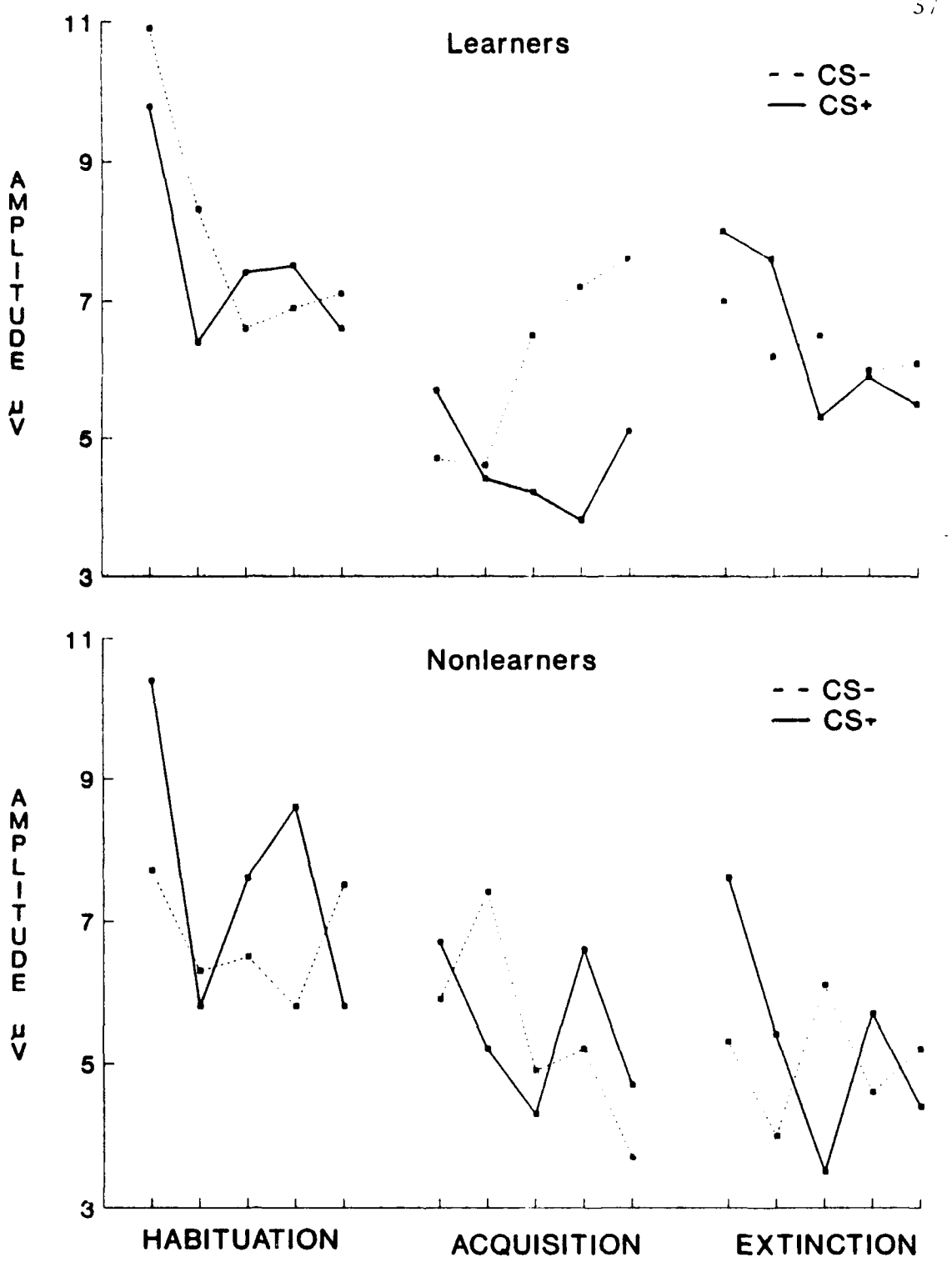


Figure 3 - P300 amplitude for learners and nonlearners across the three test phases

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6.4 Experiment 4 - Ultradian Rhythms in Performance and Evoked Potentials

Ultradian rhythms are biological rhythms with frequencies greater than one per day. Research has focussed on URs with a period in the range of 60-140 minutes. This study assesses ultradian rhythmicity in event-related cortical activity and measures of human performance. Specifically, the following questions are addressed:

(1) Are there URs in ERPs?

(2) If so, what is the relationship between URs in ERPs and performance?

As indices of state-related changes in CNS functioning, the discovery of URs in ERPs would be of interest in itself. Additionally, the demonstration of rhythmicity in ERPs may provide information regarding the CNS processes underlying performance variability. Further, a relationship between rhythms in ERPs and performance might indicate their usefulness as a predictive tool in evaluating performance readiness.

URs have been found in a number of behavioral and physiological variables. Their relationship, however, is not clearly understood. Because they are viewed as indices of such constructs as arousal, attention, and cognitive processing, ERPs are well-suited as a psychophysiological measure for the investigation of CNS correlates of performance variability.

To investigate the nature of the relationship between URs in ERPs and performance, a RT task is used. The traditional behavioral measure of decision latency has been reaction time, and there is research investigating ERP components (N2, P3) as indices of decision latency. Although behavioral validation of hypothesized cognitive processes associated with ERPs is important, past studies often failed to include RT measures. For those studies which did, the results have been equivocal. The relationship between P3 and RT appears to be stronger when the focus is accuracy rather that speed (Donchin et al., 1986). Ritter et al. (1979) suggested that the decisional processes associated with RT may have a stronger relationship to the N2 component. Globus and Lavie (1980) found no rhythm in movement time, when accuracy rather than movement time was the focus. They suggested that only task parameters which are attended will reveal ultradian rhythmicity. To investigate state-related changes in responding, this study attempts to maximize the normally occurring biological rhythm by placing no special emphasis on either speed or accuracy. The focus of the study is the N1, N2 and P3 components. N1 has been shown to be influenced by general levels of alertness (Naatanen & Picton, 1987), and several studies have shown a relationship between N1 amplitude and RT (e.g., Bostock & Jarvis, 1970; Naatanen & Gaillard, 1974). N1 amplitude was larger when RT was shorter. The other two components are considered to be influenced by alertness, attention, and cognitive processing. Like N1, N2 amplitude has also been correlated with RT, but in a reverse

manner. Larger N2 deflections were associated with longer RTs (Wilkinson & Morlock, 1966). N2 latency has also been correlated with RT (Ritter et al., 1979). The P3 component has been shown to reflect state-related changes (e.g., Broughton & Aguirre, 1987) as well as demonstrate a relationship with RT measures (e.g., Ritter et al., 1972).

METHOD

Subjects Eight male subjects, ages 18-30 (mean=24.5), were recruited from the student population of the University of Southern Mississippi. Subjects were screened for cigarette smoking, medication use, health problems, excessive caffeinated-beverage consumption, or ill effects (e.g., headaches) due to abstention from caffeinated beverages (see Appendix A for subject interview questionnaire). Subjects were paid $75 for their participation in the study.

Apparatus

Event-related potentials Subjects were tested in a 9 ft x 13 ft room. Grass gold-cup electrodes were attached to the scalp with electrode paste (impedances not exceeding 5,000 ohms). ERPs were recorded from Fz, Cz, and Pz locations (International 10-20 Electrode Placement System; Jasper, 1958) and referenced to linked mastoids. EEG was recorded from C4 and O1 and observed for signs of sleepiness in accordance with standard sleep scoring methods (Rechtschaffen & Kales, 1968). EOG was also recorded for subtracting eye-blink artifact from the averaged evoked potential measures by a computer algorithm (Gratton, Coles, & Donchin, 1983). Auditory stimuli were presented binaurally through headphones, using a Bernoulli series of randomly occurring tones. Target tones (p=.10) were set at 2000 Hz frequency and nontarget tones (p=.90) at a frequency of 1000 Hz, with a tone duration of 50 msec and a rise/fall time of 5 msec. Stimulus intensity was 60 dB (SPL), and the interstimulus interval was one second.

Tone presentations were controlled by Coulbourn Instruments solid state equipment. The EOG and the EEG signals for the ERPs were amplified by Coulbourn High-gain Bioamplifiers with high-pass filters set at 1 Hz, low-pass filters at 40 Hz, and gain at 10,000. Amplified signals were digitized and stored at 200 Hz on a Compaq 386-25 microcomputer using a Data Translation DT2821 A-D board. EEG signals for monitoring sleepiness were recorded on a Grass Model 78-D polygraph.

Reaction time An AT&T 6300 microcomputer recorded reaction times using a Data Translation DT2801 A-D board. The computer was programmed to poll the A-D channel at a rate of 200 Hz which resulted in a maximum error of 5 msecs in detection of a response. Reaction time was measured by a device strapped to the preferred hand of the subject. The device consisted of a board with straps to hold the subject's hand in a position of performance readiness and photobeam and photoreceptor cells positioned above the subject's fingers. All but the subject's index finger was secured by a strap. Upon detection of the target tone, the subject lifted his index finger to break the photocell light beam. This triggered the recording of reaction

time by the computer.

Procedure

Subjects were tested on weekends and holidays in a room free of all time cues (i.e., no clocks, watches, or windows, and a constant level of light). In addition, subjects were not informed of time elapsed or time remaining in the test period. Subjects reported to the Sleep Research Laboratory the day prior to testing, at which time experimental procedures were explained, written consent (see Appendix C) was obtained, and three practice sessions were administered. Subjects received the following instructions:

You will be listening to a series of low and high- pitched tones. You are to ignore the low tones and pay attention only to the less frequent, high-pitched tones. When you hear the high tone, count it - not out loud, but to yourself mentally - and at the same time lift your index finger as quickly as you can to break the photobeam. While you are performing the task, keep your eyes open, focus on a spot in front of you and try not to blink during tones. If you feel the need to blink, try to blink between tones. However, don't worry about blinking so much that you cause your eyes to bother you. This will only increase your desire to blink. At times you may feel drowsy. Do not fall asleep; try to remain awake, pay attention, and respond as quickly as you can. If you are having difficulty staying awake, you will be prompted to stay awake and continue responding to the high tones. In addition, subjects were instructed to refrain from alcoholic beverages the night before testing, to get a good night's sleep, and to refrain from eating breakfast or drinking coffee prior to reporting for testing, as food would be provided in the lab.

The day of testing subjects received a wake-up call from the experimenter an hour prior to reporting for the experiment. Subjects reported individually to the Sleep Research Laboratory at 08:30. After electrode hookup the subject was seated in a recliner chair in the testing room, experimental procedures were reviewed, and testing began. The total testing period lasted for eight hours with each trial commencing every 15 min and lasting approximately 5 min. The total number of tones presented in each trial was sufficient to obtain 30 randomly-presented target tones at 10 percent probability (i.e., approximately 300 total tones). RT and ERP measures were recorded for both target and nontarget stimuli and stored on computer for later off-line analyses. EEG was recorded on paper.

A constant routine (Minors & Waterhouse, 1984) was maintained throughout the testing period. It consisted of the subject remaining in the same semi-reclining position, arising only when necessary. Small, measured amounts of food and non-caffeine liquids, chosen in advance by the subject from a limited menu (see Appendix D), were provided to the subject at regular hourly intervals. Between trials, in addition to eating, the subject read selected materials, studied, or talked with the

experimenter or research assistant. During each trial EEG was monitored for signs of sleepiness, and when they occurred, the subject was prompted, at the beginning of the next trial, to stay awake and pay attention to the task.

RESULTS

ERPs After correction of each individual evoked potential using a computer algorithm for removal of eye-blink artifact (Gratton et al., 1983), both target and nontarget ERPs were averaged to obtain 33 separate waveforms (corresponding to the 33 15-min time intervals of the study) for each of the three electrode placements (Fz, Cz, and Pz). Averaged ERPs were then baseline-adjusted by subtracting the average of the first 30 points (a 150 msec prestimulus baseline) from each of the remaining 170 points. From this adjusted waveform, peaks were then scored by a computer program which picked the lowest or highest peak within a specified time window. Although the focus of the study is N1, N2 and P3 components, all peaks were scored for latency and amplitude using the following windows: P1 - 10-70 msec; N1 - 70-140 msec; P2 - 140-200 msec; N2 - 170-250 msec; P3 - 230-500 msec. Scored ERPs were checked for poorly formed peaks or ones which occurred outside the computer-scoring windows. These were scored by the experimenter by visual inspection. One subject (subject 5) had unusually long latencies for some components. For this subject, all peaks were scored by the experimenter using the visual scoring method. For all subjects, the P1 component was found to be often too poorly formed to reliably pick a peak either visually or by computer; therefore, no further analyses were done on this component.

A random check of each subject's scored waveforms was made to compare the ERPs from the three leads for a general similarity in their waveforms. Overall, these were found to be morphologically similar. The data of Subject 8 (Figure 1) is representative. Amplitude was generally maximal at the Cz lead, and all further analyses were performed with the data from Cz lead only.

Eight 33-point time series per subject were constructed from the amplitude and the latency values for each of the N1, P2, N2, and P3 components, for a total of 64 series. They were plotted for visual inspection. The data of Subjects 1 and 4 illustrate the rhythmicity found in the undetrended data (Figures 2 and 3). Each subject demonstrated rhythmicity in various ERP measures, but there were often wide differences as to which measures showed rhythmicity and the frequency at which they oscillated.

Nonstationarity is indicated by the linear trend line in these figures. In some cases, however, quadratic and cubic trends were present; consequently, the time series were submitted to polynomial regression. Regression analysis was used to correct nonstationarity in the data by subtracting third degree polynomials. Residuals after removal of the polynomials were submitted to power spectral analysis (BMDP1T), which produced spectral density estimates for 17 frequencies. All spectral

density estimates were converted to percent of total variance at each frequency. Percentages at the different frequencies were averaged across the eight subjects and then plotted.

The following test for statistical significance was performed on the averaged data. Based on the assumption of a randomly distributed variable, an equal proportion of variance would be expected to occur at each frequency, with the exception of the zero and the fundamental frequencies (0.0 and 2.9 cpd). These frequencies are associated with trends and curvilinearity in the data that was removed prior to spectral analysis by subtracting third degree polynomials. An equal proportion of variance for each of the remaining 15 frequencies was calculated to be 6.667 percent for a random variable. Next, the actual proportion of variance at the five frequencies which correspond to the period of Kleitman's (1967) BRAC (i.e., 62-124 min or 11.6-23.3 cpd) was summed. In this theoretical band of interest, the proportion of variance that might be expected to occur with a randomly distributed variable was approximately 33.33 percent (5 X 6.667). The *t* statistic was used to test whether the proportion of variance actually occurring in the BRAC frequency band was significantly different from the 33.33 percent expected to occur randomly. This procedure was performed separately for all of the ERP measures. Table 1 presents the averaged percent of variance accounted for at the 17 frequencies for the ERP measures and the mean RT measure, which is discussed separately below.

Latency Figure 4 presents the variance spectra for the latency measure for N1, P2, N2, and P3 components. The variance accounted for within the theoretical band of interest was significantly greater than expected by chance for N1 latency (40.5 percent; $t[7]=6.11$, $p<.0005$) and N2 latency (42.7 percent; $t[7]=5.601$, $p<.0005$). The proportion of variance accounted for within the theoretical frequency band was not significant for either P2 latency ($t[7]=-1.065$, $p>.05$) or P3 latency ($t[7]=-4.472$, $p>.05$). However, in several cases spectral peaks in the P3 latency data of individual subjects indicated rhythmicity. For example, Subject 8 had 54.4 percent of power in the range of 45-71 min. Other subjects had power split between primary and secondary peaks, few of which were in the theoretical band (e.g., Subject 6 with peaks at 165 and 50 min).

Amplitude Figure 5 presents the variance spectra for the amplitude measure for N1, P2, N2, and P3 components. The proportion of variance in the theoretical band for N1 amplitude was significant (36.7 percent; $t[7]=2.92$, $p<.02$), as was that for N2 amplitude (35.5 percent; $t[7]=2.124$, $p<.05$). The proportion of variance found in the BRAC frequency band was also significant for P2 amplitude (35.4 percent; $t[7]=2.35$, $p<.05$). As with the latency measure, P3 amplitude was not significant averaged across subjects ($t[7]=1.736$, $p>.05$), but the data of individual subjects showed rhythmicity. Four subjects demonstrated rhythmicity at the BRAC frequency, while the data of the other subjects reflected faster activity. For example, Subject 8 had

57.3 percent of the variance within the 62-124 min band, while Subject 3 had 52.5 percent of the variance in the 45-71 min range.

Reaction Time Due to equipment problems RT was not recorded for every target. The problem was determined to have occurred randomly, however, and therefore not deemed to bias the reaction time data. The mean number of RT measures available per trial for averaging was 16.

Three measures of reaction time for each trial were calculated: (1) mean RT; (2) the mean of the five slowest RTs; and (3) the median RT. Median RT showed very little variability; consequently, no further analyses were performed with this measure. Two 33-point time series per subject were constructed from the averaged RTs and the average of the five slowest RTs and plotted for visual inspection. The data of subjects 3 and 5 illustrate the rhythmicity found in the undetrended data for the averaged RT measure (Figure 6).

The 16 time series for RT and 5 slowest RT were corrected for nonstationarity by subtracting third degree polynomials. As with the ERP data, third order residuals were submitted to power spectral analysis, producing spectral density estimates for 17 frequencies. Finally, spectral estimates were converted to percent of total variance accounted for at each frequency (see Table 1).

Figure 7 presents the spectra for the mean of all RT scores and the mean of the five slowest RTs. The two spectra are essentially the same; however, there is more power at the BRAC frequency for the mean of all RTs (41.6% compared to 38.1%). As no additional information is provided by the mean of the five slowest RTs, no further analyses were performed with this measure. Percent of variance accounted for within the theoretical band (11.6-23.3) for the mean RT measure was tested for significance using the t statistic in the manner described above with the ERP measures.

ERP/RT Relationship Prior to analysis of the ERP/RT relationship visual inspection of the ERP and RT spectra revealed, in addition to the rhythmicity within the theoretical range, some relatively fast activity in most of the ERP measures (see Figures 4-5). As this faster activity could mask the ERP/RT relationship in the band of interest, the time series were smoothed with the von Hann numerical filter prior to analysis. This filter applies a moving average across three adjacent values with coefficients of .25, .50, and .25. In addition to removing the fast cycles in the data, this also results in attenuation in the amplitude of the five frequencies in the band of interest. However, all series were attenuated equally. Figure 8 presents the frequency response curve for the filter. In the five frequencies representing the 62-124 min period attenuation ranges from eight percent (in the slowest frequency) to 38 percent (in the fastest frequency) with a mean of 22 percent.

The relationships between reaction time and the different ERP measures were analyzed by cross-correlation (BMDP2T). When

averaging the correlation coefficients across subjects, the wide variability in period and phase among subjects reduced the ERP/RT relationship that was evident in individual subjects. Consequently, cross-correlations are described in terms of individual subjects. Figures 9 through 16 present the cross-correlation functions (CCFs) at plus and minus 30 lags, arranged by subject. Coefficients at negative lags represent correlations with the ERP series lagged, and coefficients for the positive lags represent those with the RT series lagged. Table 2 presents critical r values for a two-tailed test, .05 level of significance, for lags zero through sixteen. No correlations beyond the sixteenth lag were significant. For ease in viewing the ERP/RT relationship by component, CCFs are additionally organized by ERP measures (Figures 17 through 23).

The CCFs revealed the following:

1) ERP measures were related to reaction time. Symmetrical CCFs can be seen in all subjects, often in several measures. Correlation coefficients were as high as .74, and significant correlations often demonstrated a repeating pattern within a particular measure.
2) One of the most striking findings was that of individual differences. No one ERP measure best correlated with RT. Rather, some measures demonstrated symmetrical functions for some subjects, while other measures were better correlated for other subjects.
3) Inspection of CCFs by ERP measure (Figures 17-23) suggests that the N1 and N2 components were most strongly correlated with RT. N1 in particular had well-shaped CCFs in at least six of the eight subjects for both the latency and amplitude measures. Moreover, when the latency measure did not appear to be related to RT, the amplitude measure did. With the exception of one subject (No.9), this held true for N2 as well.

The correlation coefficients at the zero lag of the CCFs represent the simple correlations between RT and the different ERP variables. These coefficients are presented in Table 3. Significance was found for the mean correlation of RT and N2 amplitude (r=.175; $t[7]$=2.035, $p<.05$). Significant one-tailed correlations in individual subjects for different measures at zero lag are also indicated in Table 3. One-tailed tests were used to assess several hypotheses generated by the ERP/RT literature. These are discussed below.

DISCUSSION

The purpose of this study was to assess ultradian rhythmicity in event-related cortical activity and in performance. In addition, it explored the relationship between the rhythms in these two variables. The following general findings are reported:

1) URs were demonstrated in various ERP measures;
2) URs were demonstrated in reaction time;
3) a relationship between rhythms in ERP measures and

rhythms in RT was demonstrated;

4) there were wide interindividual differences as to the ERP measures which best demonstrated rhythmicity and inter and intraindividual differences in the dominant periods for the various measures;
5) there were interindividual differences as to which ERP measures best demonstrated a relationship with RT, as well as interindividual differences in period and phase of the ERP/RT relationship.

Ultradian Rhythms in ERPs The rhythmicity in both latency and amplitude for the N1 and N2 components was statistically significant. For both components, however, latency had more clearly-defined spectral peaks (Figure 4), compared to the amplitude spectra (Figure 5) where power was more widely distributed across the frequencies. For P2 amplitude, which was statistically significant, power was also widely distributed across frequencies, while the P2 latency measure was not statistically significant.

Although a significant amount of variance was not found in the theoretical band for either latency or amplitude in the averaged spectral values for the P3 component, inspection of the spectra for individual subjects revealed rhythmicity in many cases. P3 amplitude in particular demonstrated rhythmicity in individual subjects. Wide variability in individual spectral peaks accounted for the lack of significant rhythmicity in the theoretical band when spectral estimates were averaged across subjects. This observation highlights a sometimes problematic characteristic of time series analysis.

Although averaging across subjects can improve stability of the spectral estimates, it may obliterate spectral peaks in highly variable measures. Consequently, the importance of perusing individual spectra should not be overlooked. In addition to indicating rhythmicity that would be lost in the averaging process (i.e., as with P3), inspection of individual spectra also revealed that averaging often greatly reduced spectral power for the various measures. For example, with Subject 7 the amount of variance accounted for in N1 amplitude in the 62-124 min band was greater than 55 percent, while averaging across subjects resulted in 36.7 percent. Some subjects demonstrated similarly strong rhythmicity outside the BRAC frequency band. For example, N1 amplitude for Subject 4 had 57.4 percent of the variance within the 41-62 min range. Individual inspection also revealed that in addition to power at primary peaks, some subjects had secondary peaks. It is difficult to interpret whether these additional peaks are bona fide secondary periodicities or the result of deviations in the data from a purely sinusoidal shape, 'leakage' from the slower frequencies, or the power from faster frequencies which lie outside the sampling rate of the analysis and are 'aliased' to frequencies within the bounds of the analysis (Bloomfield, 1976). Of interest is the temporal relationship between the various ERP component measures. A range of 60 to 120 min best

described the BRAC period as discussed in the ultradian literature. Consequently, statistical tests for significance were done on the basis of 62-124 min (the corresponding periodicity represented in these data). However, these boundaries represent tendencies in the data, and both slower and faster activity have been reported. While the temporal center of the BRAC period for this study was approximately 83 min, all of the ERP measures which showed significant power in the theoretical band had spectral peaks indicating faster rhythms. N1 latency and amplitude, P2 amplitude, and N2 latency all peaked at 71 min. N2 amplitude had a 55-min peak, i.e., outside the theoretical window. While P3 latency was not statistically significant within the BRAC band, it had, in addition to a nonsignificant peak at 165 min, a significant peak at 55 min ($t[7]=1.933$, $p<.05$).

To some extent this difference in dominant peaks may be determined by the limits of the statistical procedure. There is poor resolution between the frequencies at the 'slow end' of the spectrum when the number of points in the time series is relatively small. In this study, for example, power could be expressed at either 83 or 99 min when in reality oscillations may have resembled more closely 90 min cycles. However, given this caveat regarding statistical limitations, it is noteworthy that all ERP measures demonstrated activity somewhat faster than the average BRAC frequency. This difference may reflect the fact that ERPs are a measure of CNS activity. Most measures demonstrating ultradian rhythmicity have been peripheral psychophysiological measures, such as heart rate or urine composition, or performance measures, which involve both central and peripheral mechanisms. ERPs are more closely related to the initial processes involved in perceiving and evaluating stimuli.

Of further interest are the differing periodicities within the ERP measures themselves. N1 latency, N1 amplitude, P2 amplitude, and N2 latency demonstrated the same 71-min periodicity, while N2 amplitude and P3 latency had periods of 55 min. It is possible that these differences reflect the exogenous/endogenous component dichotomy. There are several characteristics which differentiate these categories. The exogenous components (N1, P2) are more closely related to sensation and perception and are far less variable in latency than the later endogenous components (N2, P3), which are considered to be indices of information processing. Comparing, for example, N1 and P3 we find that N1 latency typically occurs within a 50-msec window, while the latency window for the P3 component is approximately 300 msec. With stimulus characteristics held constant, the major factor influencing N1 is subject state. In other words, URs may appear as fluctuations in overall level of arousal. The P3 component, on the other hand, is additionally influenced by psychological factors such as attention and motivation.

Although N2 is typically identified as an endogenous component, N2 latency had the same period as the exogenous

measures. The 'coupling' of the N2 latency measure with the N1 component may be the result of the MMN. The MMN, elicited by a deviant stimulus, is a broad component that can overlap both the N1 and N2 components. While the MMN is not affected by attention (an endogenous factor), it does vary with differences in arousal level (Sams, et al., 1983). The discovery of URs in ERPs with awake adult subjects is an important finding with respect to rhythmical variations in event-related potentials. Due to the paucity of research in the area, no direct evidence of URs in ERPs existed for this population. Previous research either demonstrated diurnal variation with sampling rates insufficient to establish ultradian rhythmicity (Broughton & Aguirre, 1987; Harsh & Badia, 1989), or changes in ERPs during sleep (Tanguay et al., 1973) or immediately following arousal from different stages of sleep (Broughton, 1968). If the cyclicity of REM sleep is a sleep-dependent phenomenon as purported by Moses et al. (1977), rather than the sleeping manifestation of an ongoing BRAC (Kleitman, 1963), then the findings of URs during sleep cannot be generalized to waking populations. The generalizability of results from ERPs collected immediately following arousals from sleep is also questionable. In addition, subjects were either children, narcoleptics or sleep-deprived individuals, making generalization from these earlier studies to a normal adult population impossible.

In addition to the demonstration of ultradian rhythmicity in waking ERPs, the present study also provides confirmation of the slower diurnal trends that have been seen in previous studies (Browman, 1979; Harsh & Badia, 1989). Although the data were detrended prior to spectral analysis because the focus of this study was ultradian rhythmicity, diurnal trends were present in the data of most subjects (see Figures 2 and 3).

Ultradian Rhythms in RT RT clearly demonstrated ultradian rhythmicity in this study. Power spectral estimates were more stable for this measure than most of the ERP measures, with all subjects having either primary or secondary peaks within the BRAC band. In addition, the period for RT was consistent with the BRAC literature on performance measures, as evidenced by the clearly-defined spectral peak at 99 min (Figure 7).

These data support Lavie's (1982) observation that task parameters and experimental design may play a role in the manifestation of ultradian rhythmicity. Lavie's adaptive serial RT task and the provision of feedback in the Gopher and Lavie (1980) study seemed to modulate rhythmicity in performance. This study, on the other hand, replicated the findings of Stampi and Stegagno (1985) using the cognitively more demanding CRT task, as well as a protocol which held stimulus characteristics constant and placed no special emphasis on speed or accuracy. This lack of emphasis may account for the emergence of the relatively 'delicate' ultradian rhythm. Given the influence of experimental design, the stability of this measure compared to most of the ERP measures is notable.

ERP/RT Relationship Although a relationship was demonstrated

between changes in ERPs and changes in RT, the nature of the relationship was complex, with individual differences being a striking feature of the data. Overall, the N1 and N2 components seemed to be most strongly related to changes in performance. For N1 latency (Figure 17), seven of the eight subjects, and for N1 amplitude (Figure 18), six of the subjects, had fairly symmetrical CCFs. For N2 latency (Figure 19), five subjects, and for N2 amplitude (Figure 20), at least six subjects, had symmetrical functions. In all cases, however, there was such wide variability in period and phase, coefficients could not be averaged across subjects without the loss of a significant amount of information. While the evidence is not conclusive, the strong relationship between the N1 and N2 components and RT suggests the possibility that the MMN is actually the component that best predicts performance. Further evidence for this conclusion may be indicated by the P2 amplitude measure which should also be influenced by the MMN due to its location between the N1 and N2 deflections. CCFs for P2 amplitude were also well-formed (Figure 21).

Although analysis of the ERP/RT relationship focussed on cross-correlation and the shape of the CCFs, several other methods provided additional information. One of these was a comparison of the ERP and RT periods. The exogenous components (N1, P2) with a period of 71 min, and the endogenous components (N2, P3) with a period of 55 min, demonstrated somewhat faster activity than RT (99 min period). Once again bearing in mind the statistical limitations of the analysis which make an exact determination of periodicity impossible, these data suggest that the nature of the measures played a role in generating different periodicities. While ERPs are measures of CNS activity involving processes such as sensing, perceiving, stimulus evaluation, and decision making, responding is dependent on a complex of sensory, cognitive, and motoric processes. That the periodicity of the CNS measures was shorter than that of RT may indicate that changes in CNS state occurred prior to changes in performance. In other words, reductions in arousal may not be simultaneously accompanied by performance deterioration. It is possible that as reductions in arousal occurred subjects were able through some compensatory mechanism, either motivational or motoric, to sustain performance levels for a time. This process may lengthen the period of the performance measure.

Another source of information was the simple correlations between RT and the ERP variables (see Table 3). Ritter et al. (1979) proposed that N2, rather than P3, latency was an index of decision-making. Under the Ritter hypothesis, N2 latency and RT should be positively correlated. These data provided some support of this claim. While the mean correlation for RT and N2 latency ($\underline{r}$=.175) was significant ($\underline{t}[7]$=2.035; $p<.05$), the mean correlation for RT and P3 latency ($\underline{r}$=.121) was not. However, for the RT/N2 latency relationship, although all but one subject had positive correlations, only three of the individual correlations were significant. For the RT/P3 latency

relationship, three subjects also had significant positive correlations, but several subjects had negative correlations.

Other findings were not supported. Several studies (e.g., Bostock & Jarvis, 1970; Naatanen & Gaillard, 1974) found that RT was shorter when N1 amplitude was larger. While four of the eight subjects in this study had negative N1 amplitude/RT correlations, only one was significant (r=-.726, p<.005), and the mean correlation (r=-.026) was not significant. Nor was a finding of a positive relationship between N2 amplitude and RT (Wilkinson & Morlock, 1966) supported by this study. Only two subjects had significant positive correlations, and the mean correlation (r=.081) was nonsignificant.

Although no literature on the relationship between N1 latency and RT was found, these data indicated a strong relationship between the two variables. By hypothesizing a positive relationship (i.e., the longer it takes to sense/perceive the stimulus, the longer reaction time should be), significant correlations were found for four subjects, but the mean correlation for all subjects (r=.139) was not significant. However, it can be seen from Table 3 that this was due to the large negative correlation of one subject (r=-.677). It is possible that this subject attempted to compensate for waning arousal by emphasizing response speed. Without this subject, the mean correlation for RT and N1 latency (r=.256) was significant ($t[6]$=3.82; p<.005).

Implications for the BRAC Hypothesis These data reflect the complexity of the processes underlying ultradian rhythmicity. They do not resolve the debate over single vs. multioscillatory mechanisms. Although the different periods in the various measures might indicate a multioscillatory answer, a single-mechanism explanation cannot be ruled out, as the different measures are related to different processes. The exogenous components, reflecting sensory processes, may be under stricter control of the biological mechanism, while the endogenous components, involving cognitive processes such as attention, may be more susceptible to distractions and/or motivational factors and therefore more loosely controlled. At the end of a chain of processes is responding. It begins with the initial sensory encounter of the stimulus and incorporates the various sensory and cognitive processes with motoric functioning. This complexity of processes in the performance measure may influence oscillatory control in various ways. In this study RT was seen to be a more stable measure with a longer period than the ERP measures.

Conclusion The results of this study indicate that a certain amount of variability observed in measures of CNS activity and performance is due to biological rhythms at the ultradian frequency. It is suggested that this rhythmicity is manifested as fluctuations in general level of arousal. In addition, these data indicate that ERPs hold promise as a predictor of performance degradation. Future research is needed

to explore this possibility. In particular the indication that the changes seen in the N1 and N2 components is the result of the MMN needs further investigation.

More information is also required to determine if differences in periodicity observed with the exogenous and endogenous components is meaningful. Because of the small number of subjects and the problem of defining periodicity, associated with relatively limited data points, conclusions with respect to this finding must be tentative. Replication of these results is needed.

The difference in period for the ERP measures and RT is an interesting finding which also needs further investigation. This finding is not only of theoretical interest but its replication might provide useful information with respect to prediction of performance from ERPs.

Finally, an unresolved statistical issue is the relationship between the number of data points and frequency resolution at the slow end of the spectrum, and its effect on periodicity. Future research should increase the data point to time period ratio in order to improve resolution. This may help to clarify questions about the periodicity of the measures. Elimination of the 'odd-ball' paradigm would reduce the time on each trial, thereby making it possible to sample more frequently within each hour. However, for studies investigating cognitive processes such as decision- making and ERPs as a predictor of performance degradation, this approach is not suitable, since the MMN and the endogenous components are generated by the odd-ball paradigm. Another way to increase sampling rate, more suitable for ERP/performance studies, is to change the target to non-target ratio from 10:90 to 20:80. This would also reduce sampling time in each trial and make it possible to sample more frequently.

On the other hand, these data indicate that elimination of the odd-ball paradigm would not pose a problem for studies focussing on ERPs as a physiological marker of the rhythm. The strong rhythmicity found in N1, which is more closely related to sensing and perceiving than to decisional processes, suggests that this component, when experimental parameters are held constant, is a good indicator of subject state and can serve as a marker of the biological rhythm. Studies investigating, for example, the relationship between ultradian rhythmicity in ERPs and other physiological measures might consider elimination of the odd-ball paradigm in order to increase sampling rate.

A final possibility is to lengthen the overall testing period. Although subjects were becoming mentally and physically fatigued towards the end of the eight-hour test period, this time could possibly be increased by as much as two hours before subjects became mutinous.

Table 1

Average Percent of Variance Accounted for at 17 Frequencies

cpd	N1Lat	N1Amp	P2Lat	P2Amp	N2Lat	N2Amp	P3Lat	P3Amp	RT
0.0	0.49	0.56	0.43	0.57	0.55	0.48	1.14	0.62	0.71
2.9	1.68	1.75	1.41	1.87	1.96	1.65	3.54	2.08	2.11
5.8	4.14	3.39	3.60	4.90	5.47	4.24	7.36	5.10	5.45
8.7	6.06	4.22	6.05	6.94	8.64	6.38	8.69	6.66	8.15
11.6	**6.99**	**5.13**	**6.93**	**6.39**	**8.82**	**6.56**	**6.94**	**6.32**	**9.10**
14.5	**7.55**	**7.04**	**6.51**	**6.04**	**8.24**	**6.38**	**5.14**	**6.48**	**9.68**
17.5	**8.87**	**8.18**	**5.94**	**7.34**	**9.16**	**6.86**	**4.85**	**7.68**	**9.38**
20.4	**9.31**	**8.28**	**6.11**	**8.25**	**9.28**	**7.52**	**6.07**	**7.68**	**7.56**
23.3	**7.78**	**8.08**	**6.46**	**7.42**	**7.21**	**8.14**	**7.42**	**6.95**	**5.86**
26.2	6.26	7.81	6.26	6.24	5.25	8.46	7.51	6.60	5.64
29.1	6.43	7.94	5.51	6.34	4.32	7.75	7.07	6.10	5.94
32.0	6.01	8.20	5.62	7.27	3.80	6.47	6.58	5.46	6.47
34.9	5.01	7.73	6.06	7.72	4.15	6.14	6.34	6.52	6.26
37.8	4.94	5.89	6.69	6.53	5.26	6.29	5.61	7.53	5.43
40.7	5.17	4.51	7.76	5.91	6.02	6.52	5.01	6.95	4.45
43.6	5.95	4.92	9.10	5.13	6.05	5.72	5.03	5.82	4.72
46.5	7.34	6.38	9.58	5.14	5.82	4.46	5.71	5.46	3.10

Boldface type represents the BBAC frequency band.

Table 2

Critical r Values for .05 Level of Significance, Two-tailed

Lag	df	r
0	31	.344
1	30	.349
2	29	.355
3	28	.361
4	27	.367
5	26	.374
6	25	.381
7	24	.388
8	23	.396
9	22	.404
10	21	.413
11	20	.423
12	19	.433
13	18	.444
14	17	.456
15	16	.468
16	15	.482

Table 3

Correlations at Zero Lag

SUB #	N1LAT/RT	N1AMI/RT	N2LAT/RT	N2AMP/RT	P3LAT/RT	P3AMP/RT
1	.088	-.106	.488*	.250	.316*	-.326*
2	.218	.259	.062	.276	.300*	-.130
3	.426*	.261	.495*	-.340	-.121	-.496*
4	.406*	.170	.307*	.404*	-.076	-.298*
5	-.052	-.185	.035	.497*	.392*	.284
6	.376*	-.726*	.161	-.595	.211	-.227
7	.327*	-.196	.254	.088	-.148	.064
8	-.677	.318	.146	.065	.096	.265
Mean	.139	-.026	.175**	.081	.121	-.108

* r is significant with 1-tail test; $p < .05$
** t is significant with 1-tail test; $p < .05$

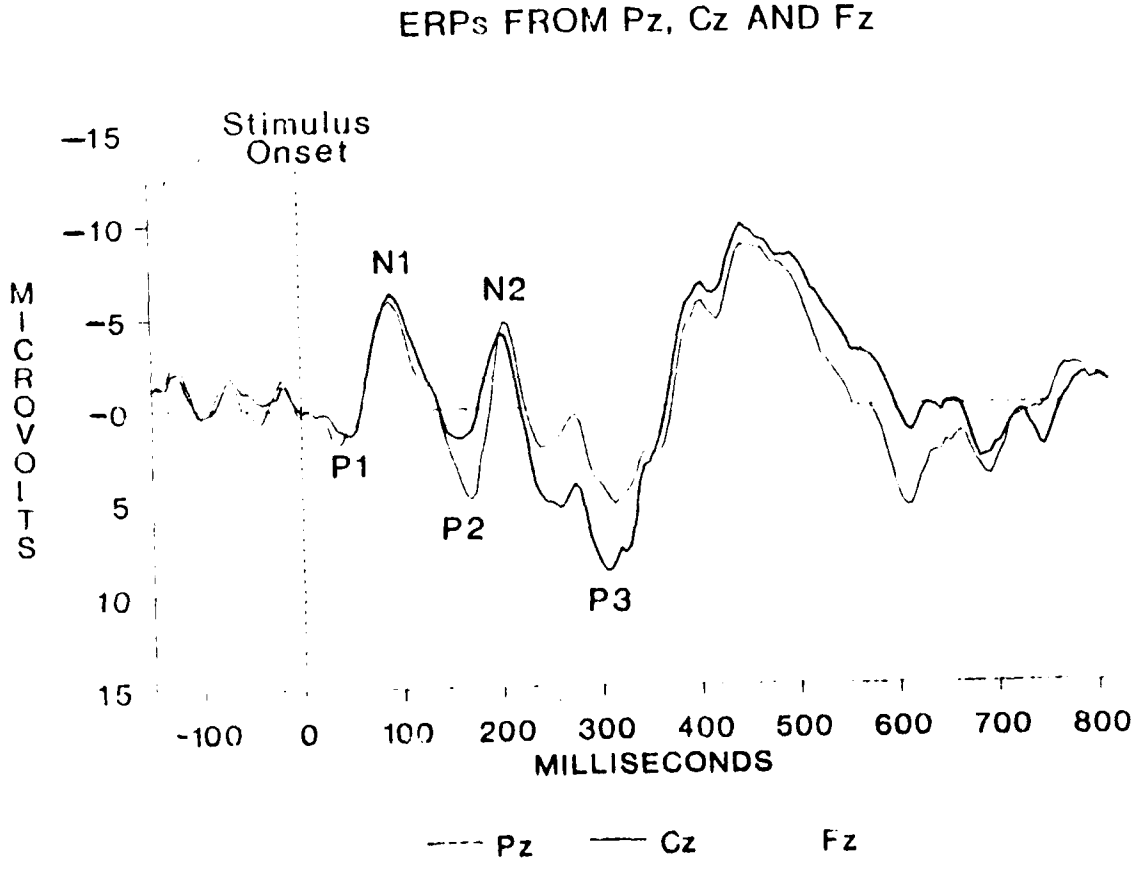


Figure 1 - ERPs from Pz, Cz, and Fz leads (Subject 8)

SUBJECT 1

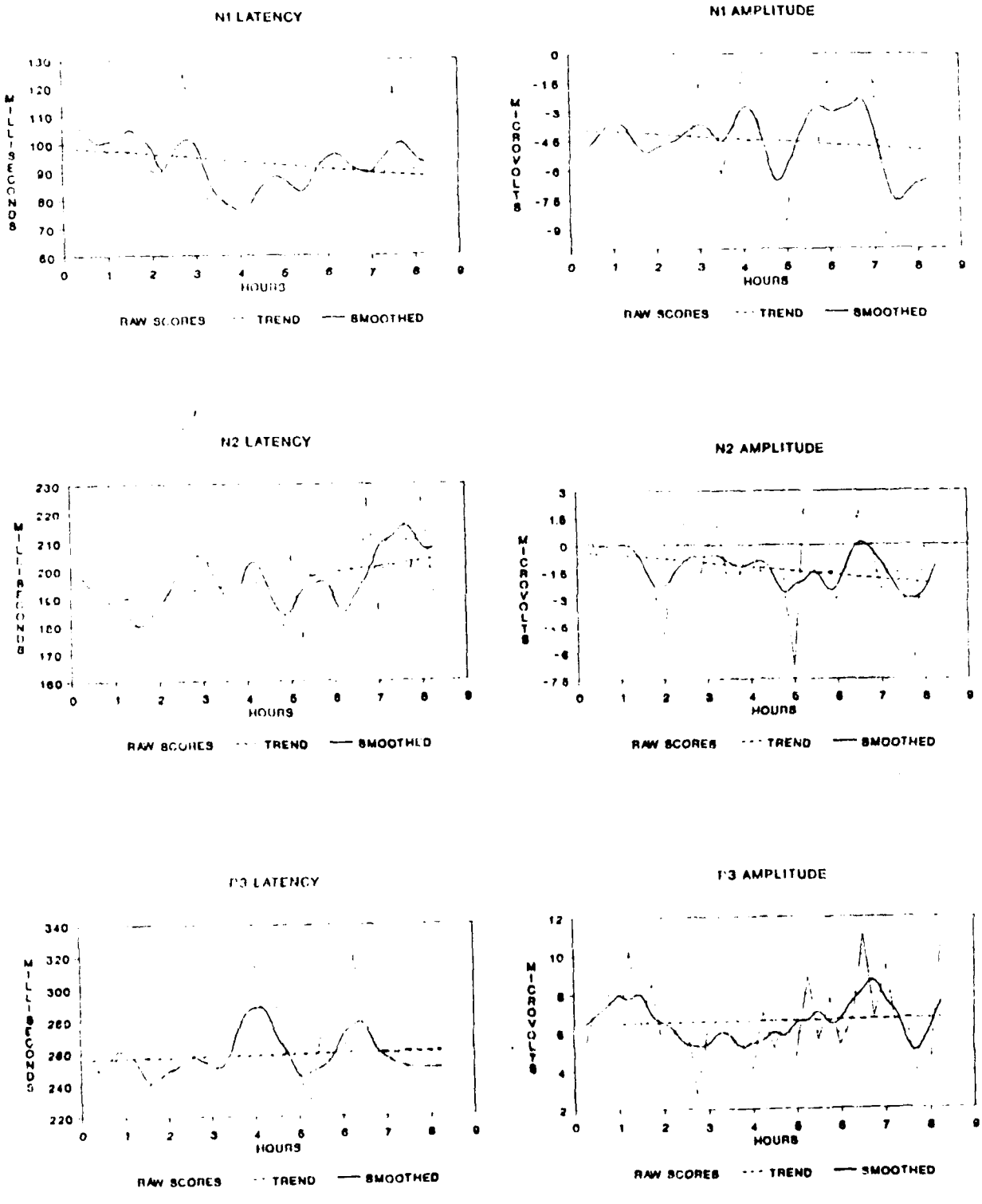


Figure 2 - Time series data for N1, N2, and P3 latency and amplitude (Subject 1)

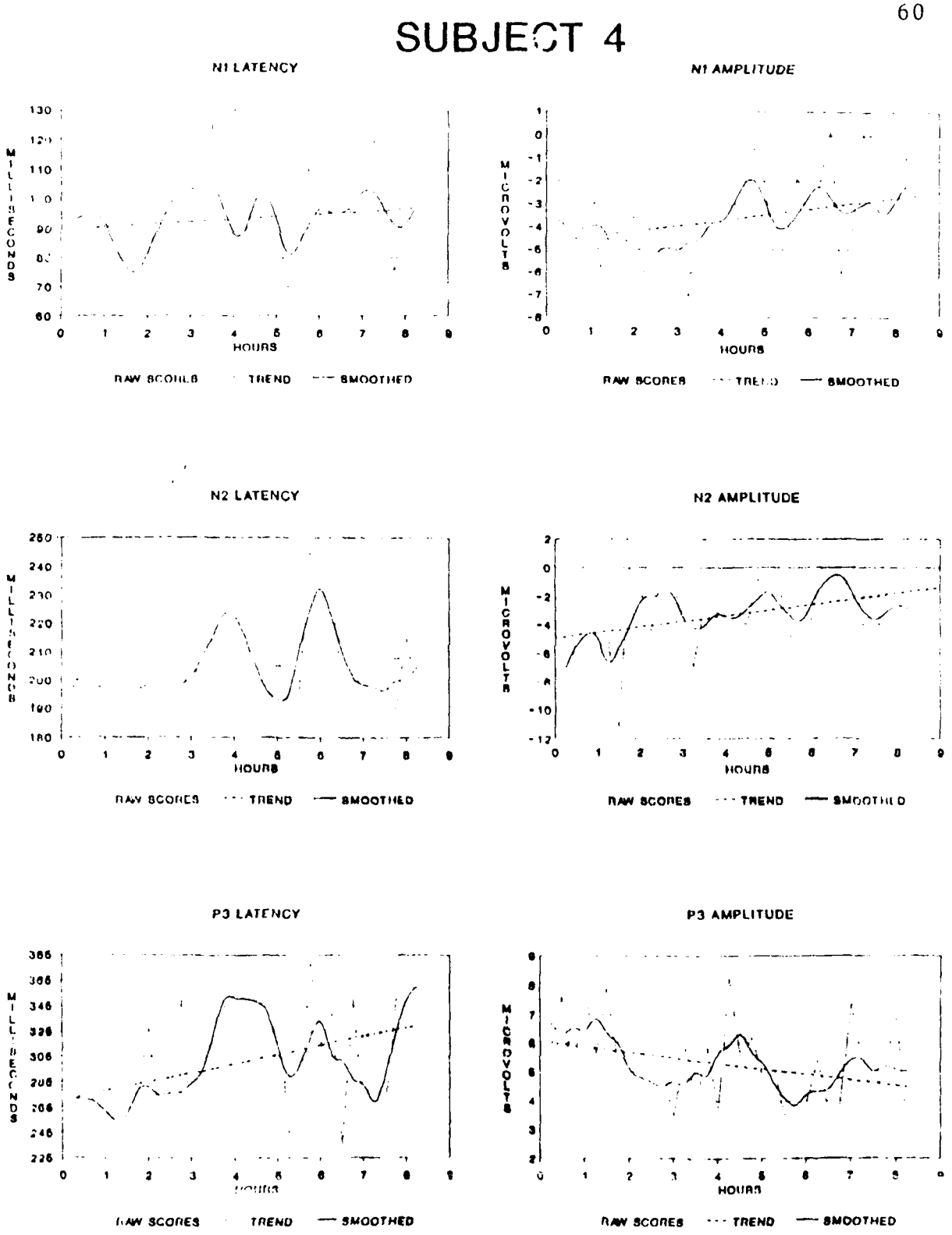


Figure 3 - Time series data for N1, N2, and P3 latency and amplitude (Subject 1)

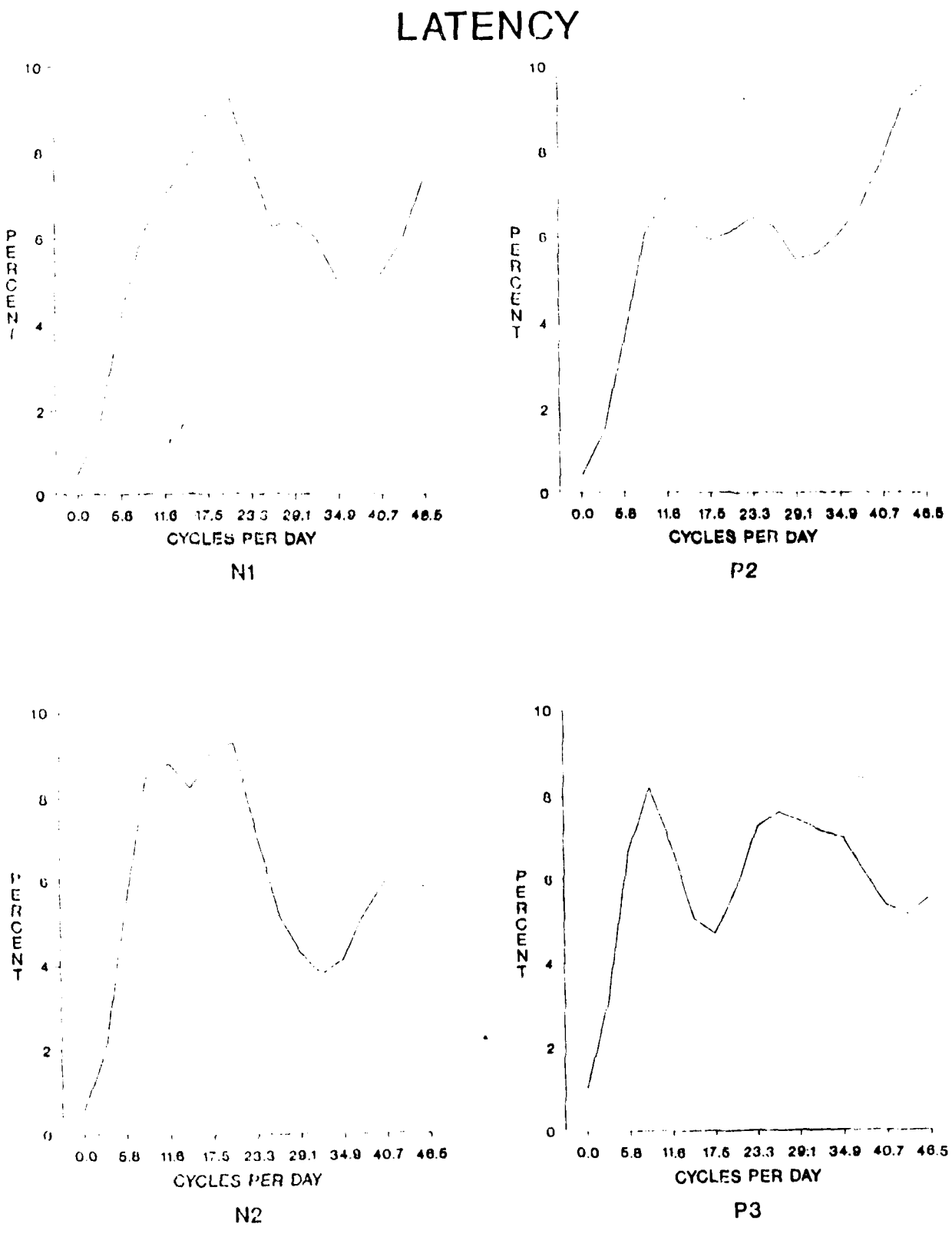


Figure 4 - Latency spectra (averaged across 8 subjects)

AMPLITUDE

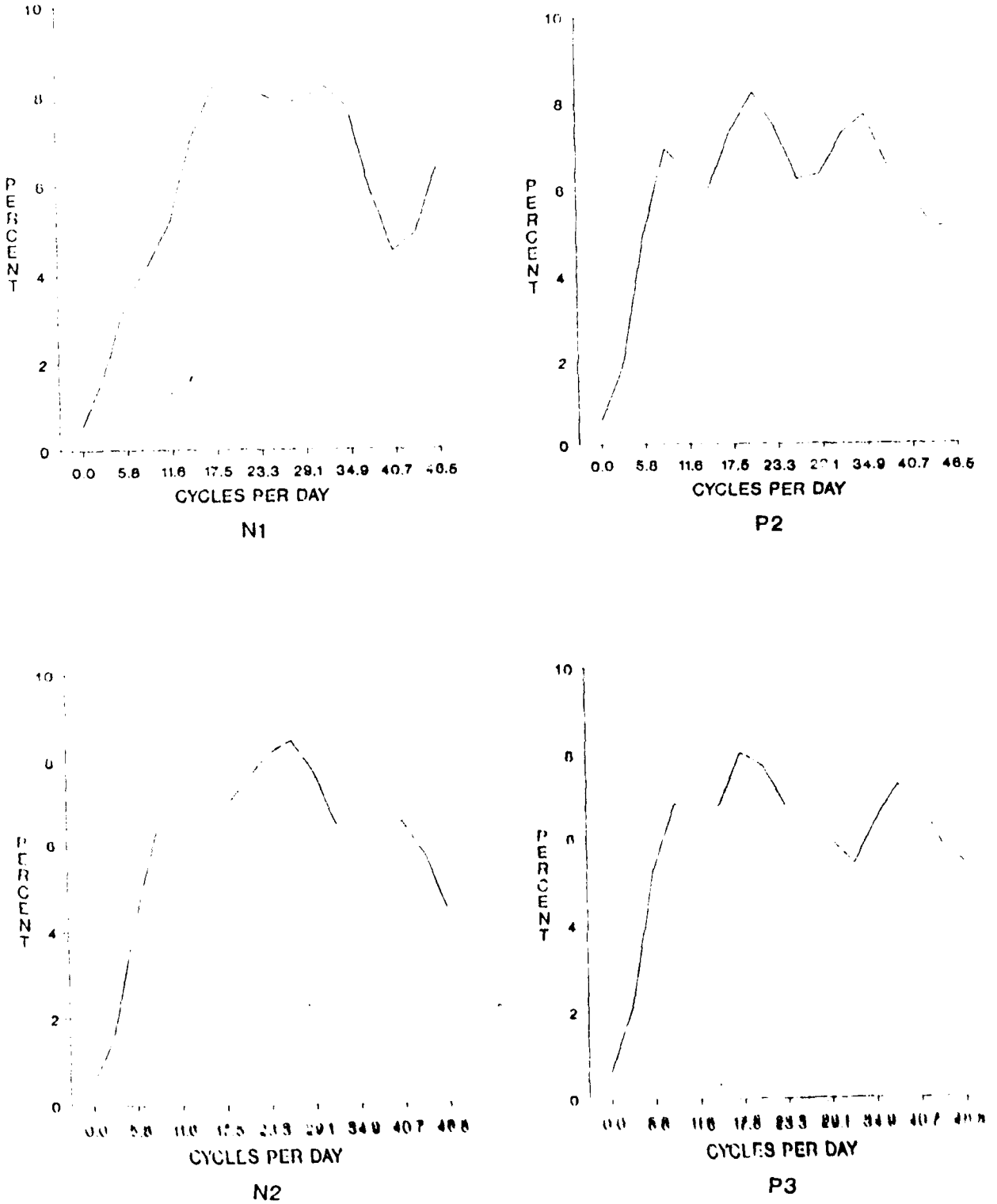
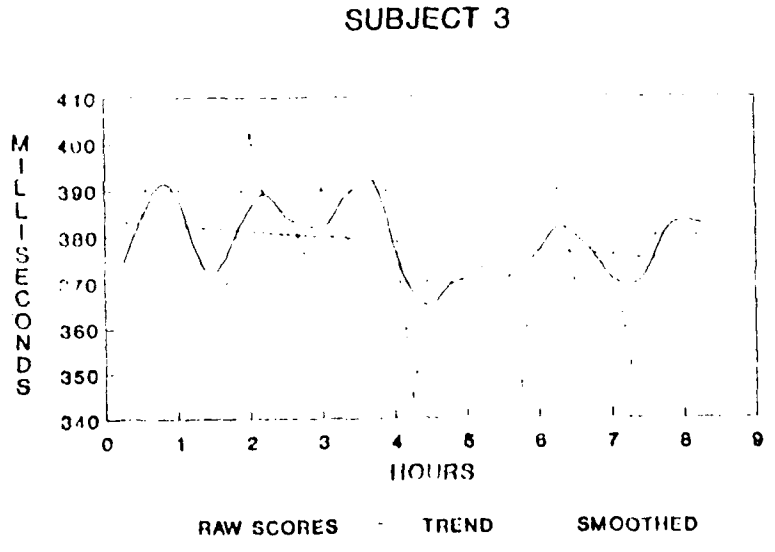


Figure 5 - Amplitude spectra (averaged across 8 subjects)

REACTION TIME



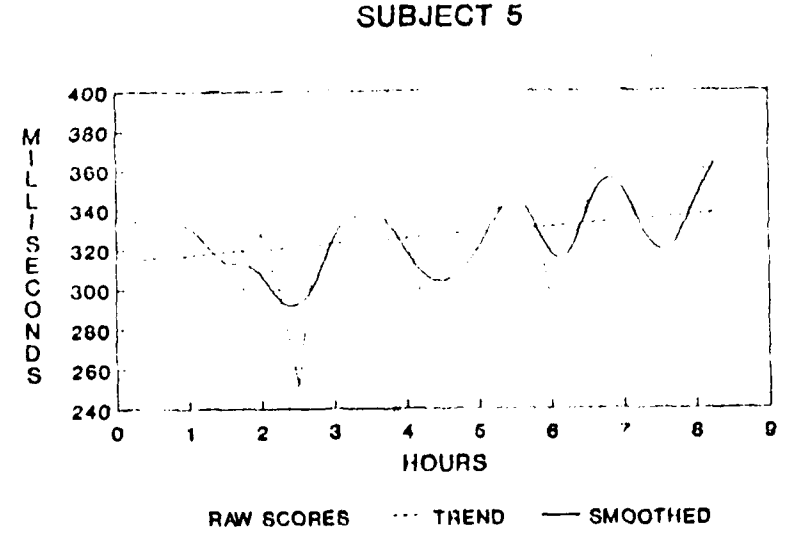


Figure 6 - Time series data for mean reaction time (Subjects 3 and 5)

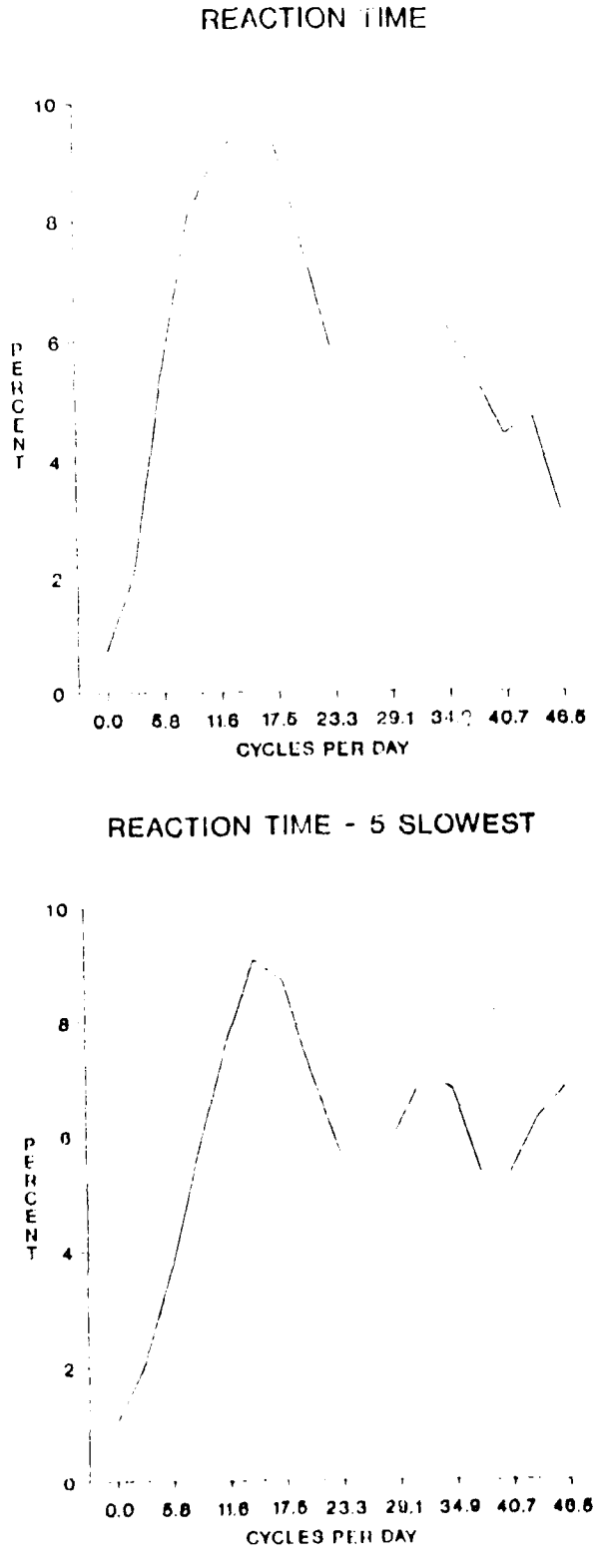


Figure 7 - Spectra for mean RT and Mean of the 5 slowest RTs (all subjects)

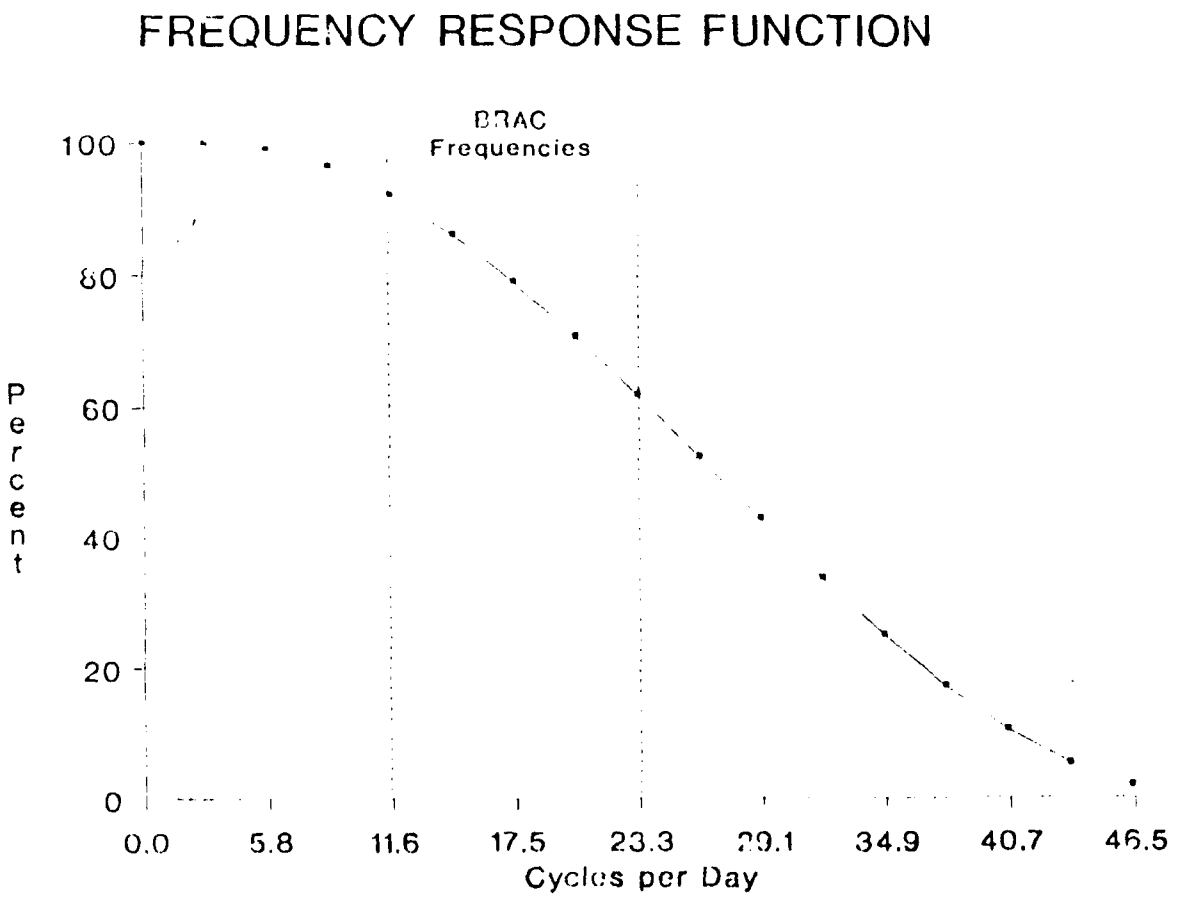


Figure 8 - Filter frequency response function

SUBJECT 1

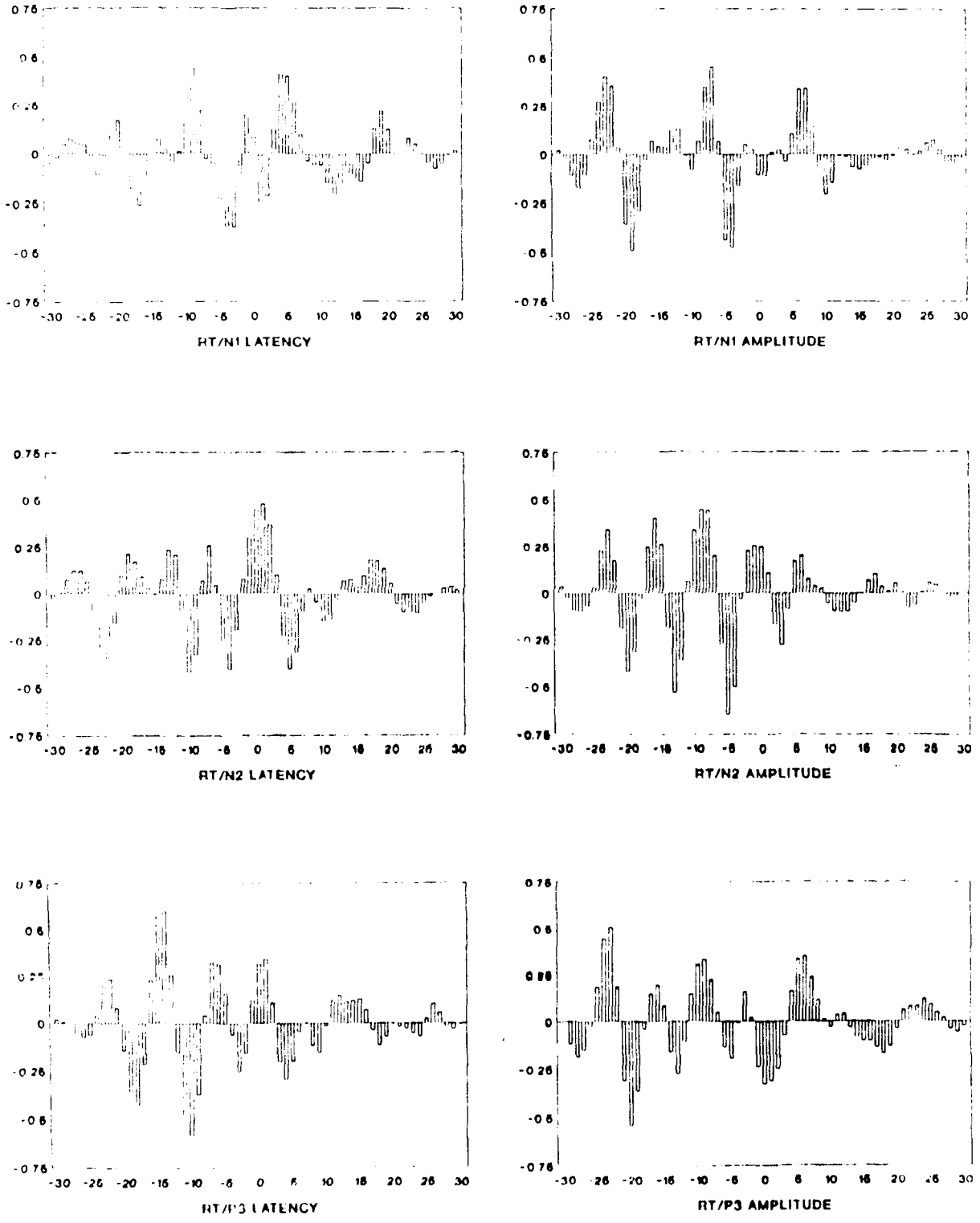


Figure 9 - Subject 1 ERP/RT Cross-correlation Functions

SUBJECT 2

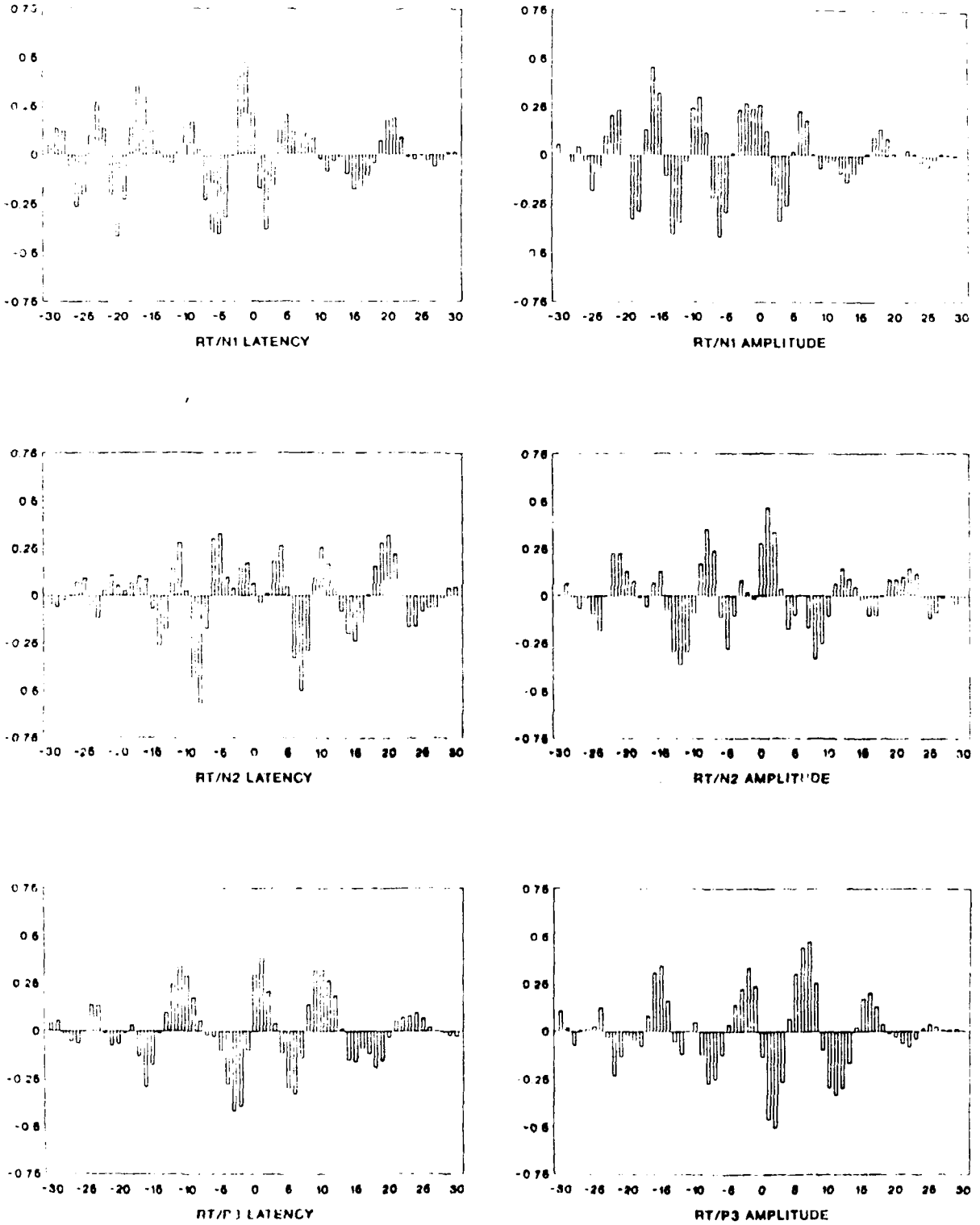


Figure 10 - Subject 2 ERP/RT Cross-correlation Functions

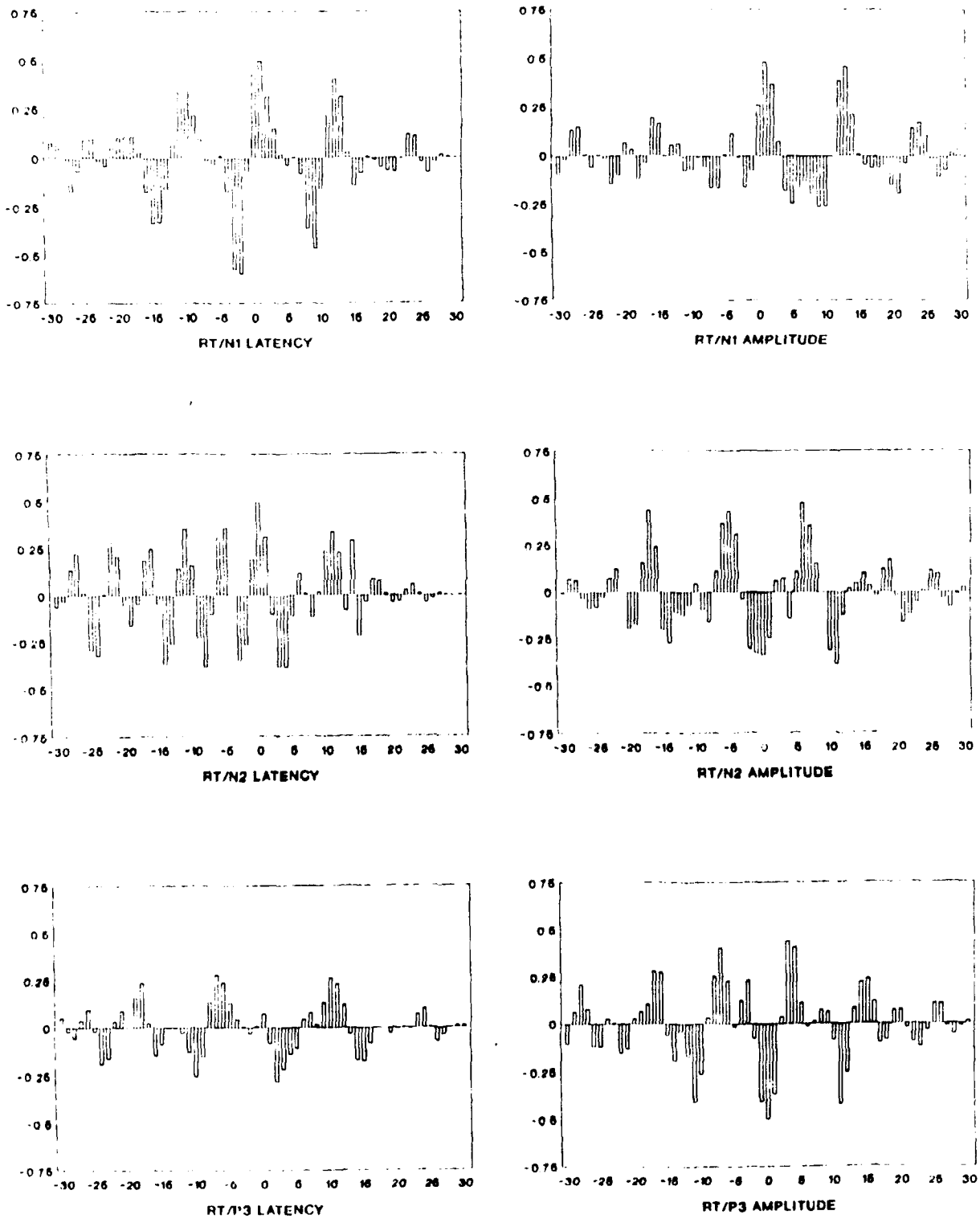


Figure 11 - Subject 3 ERP/RT Cross-correlation Functions

SUBJECT 4

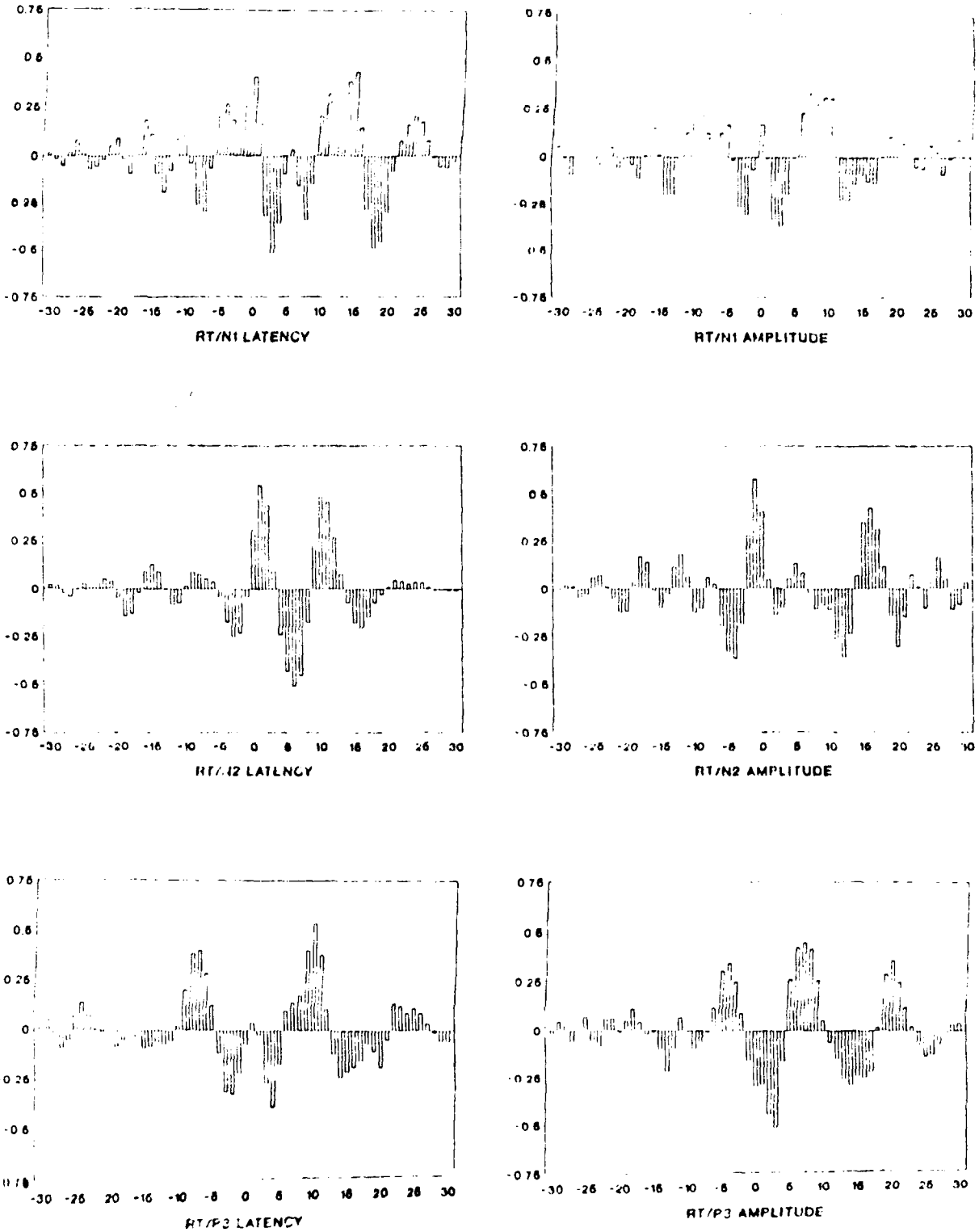


Figure 12 - Subject 4 ERP/RT Cross-correlation Functions

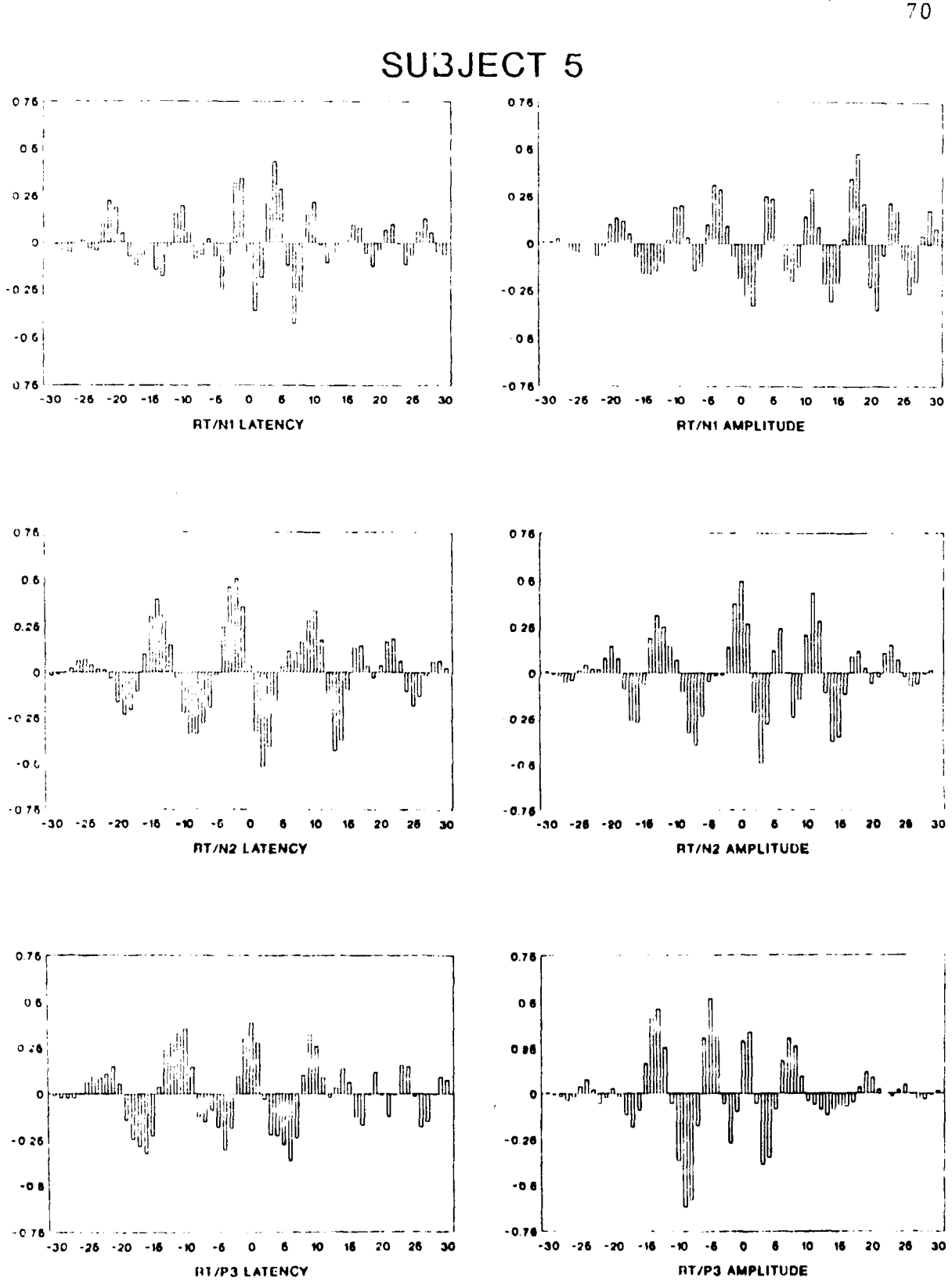


Figure 13 - Subject 5 ERP/RT Cross-correlation Functions

SUBJECT 6

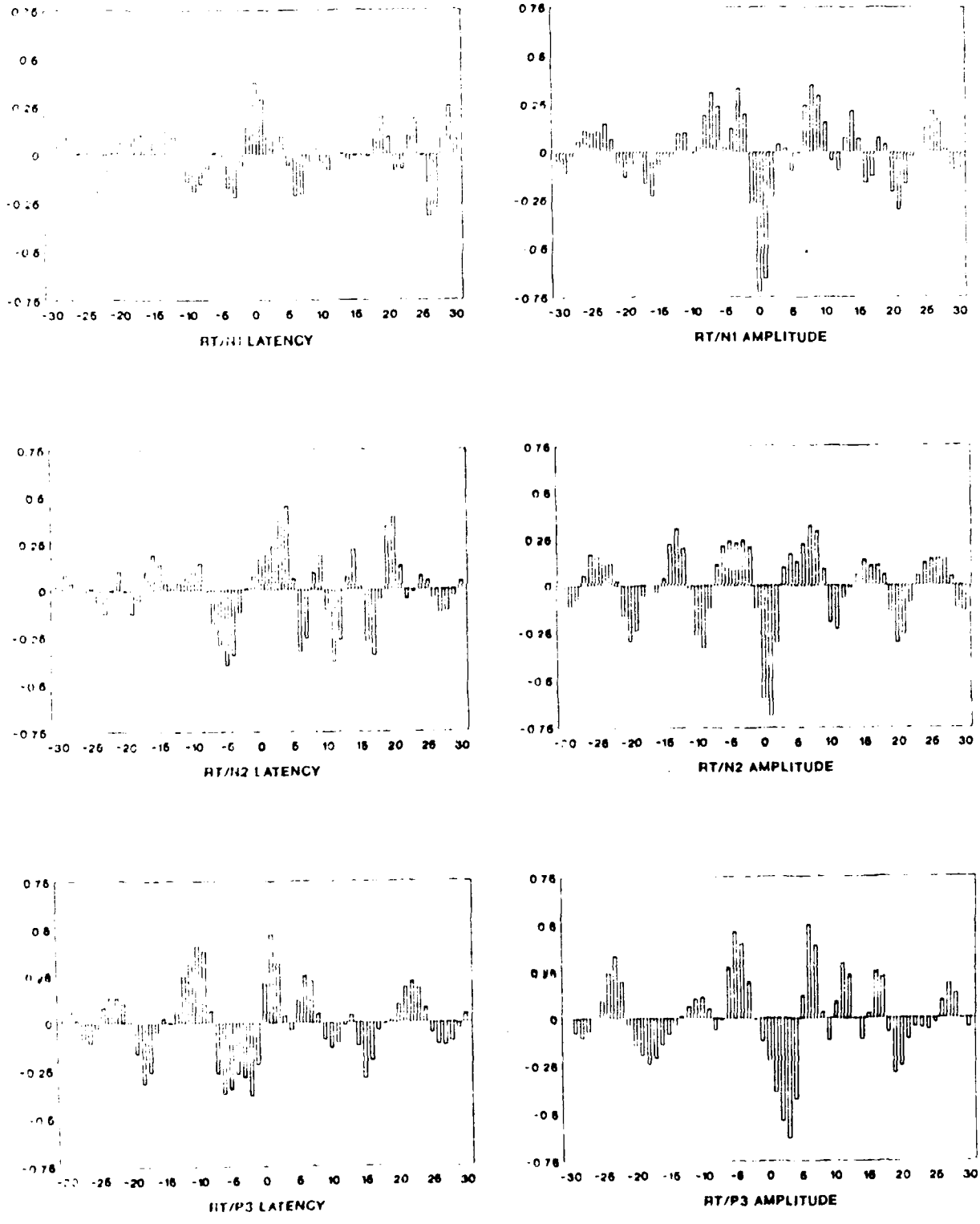


Figure 14 - Subject 6 ERP/RT Cross-correlation Functions

SUBJECT 7

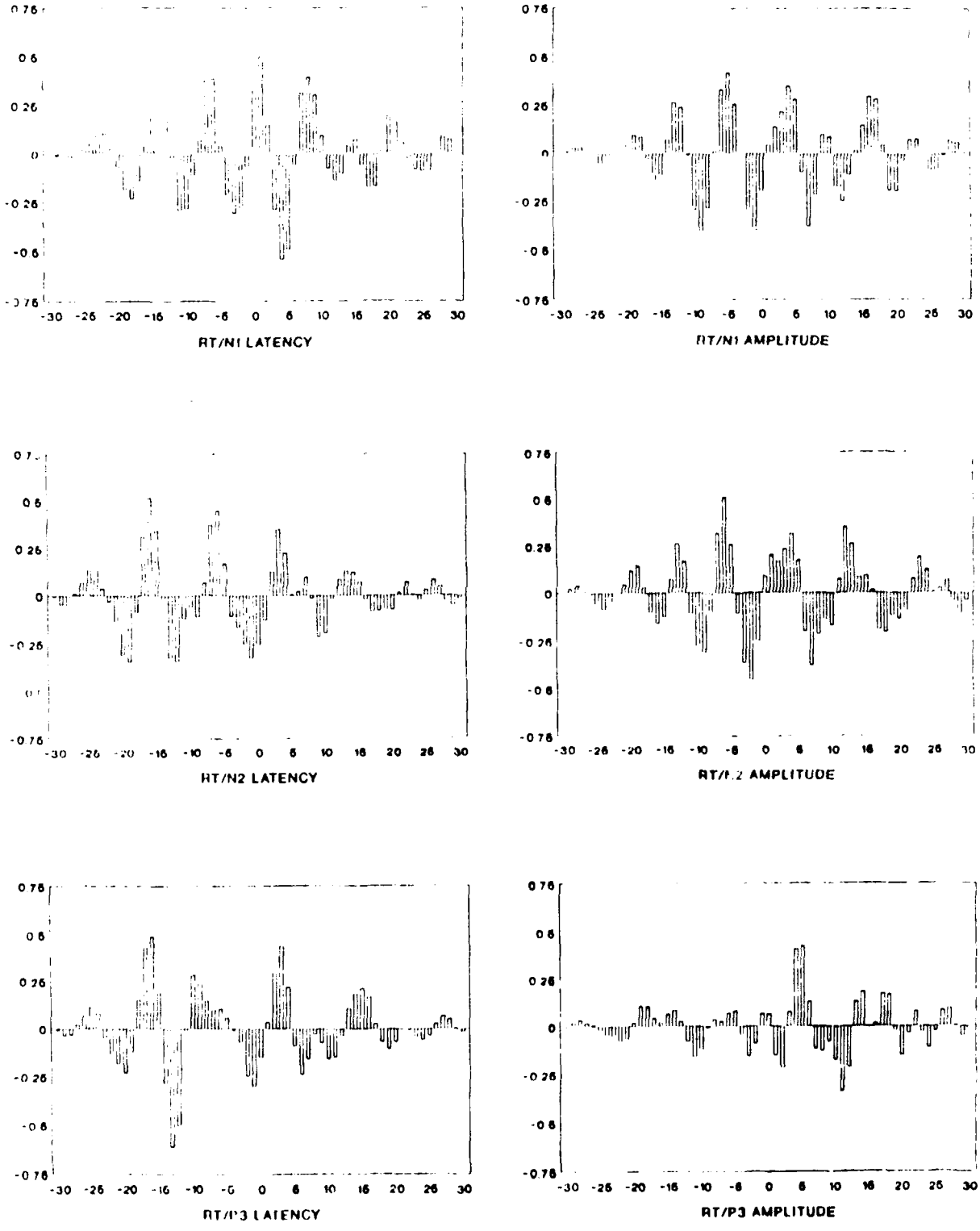


Figure 15 - Subject 7 ERP/RT Cross-correlation Functions

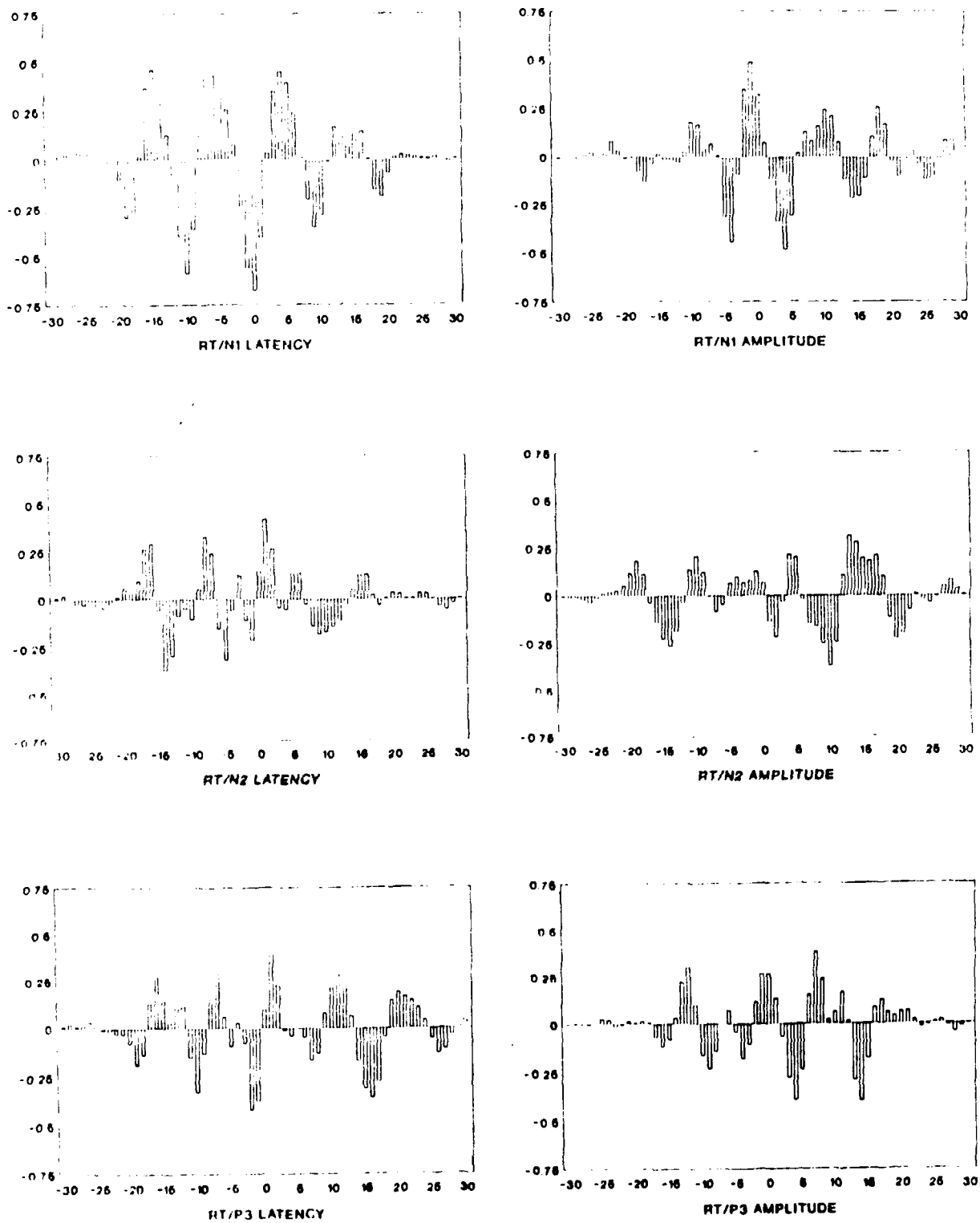


Figure 16 - Subject 8 ERP/RT Cross-correlation Functions

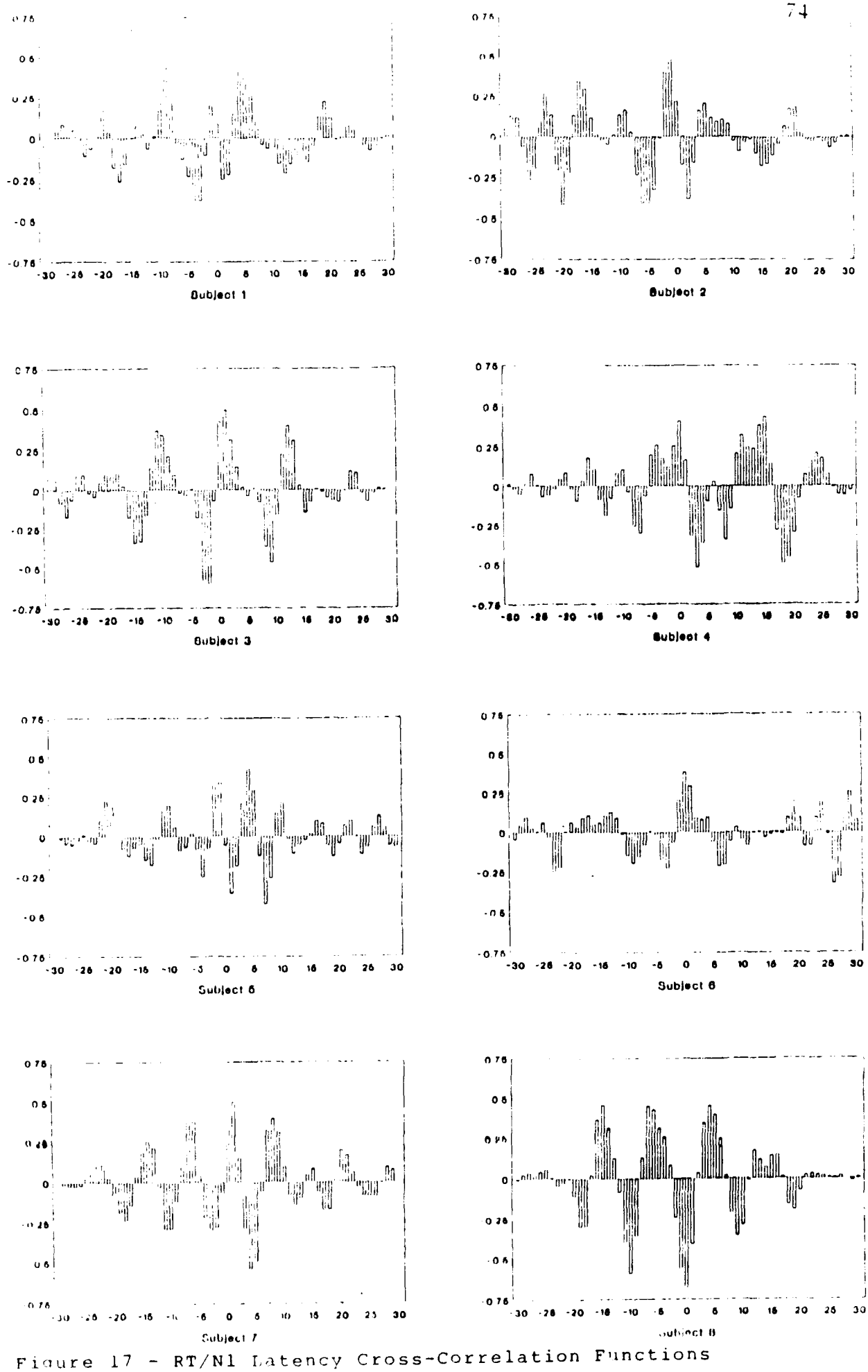
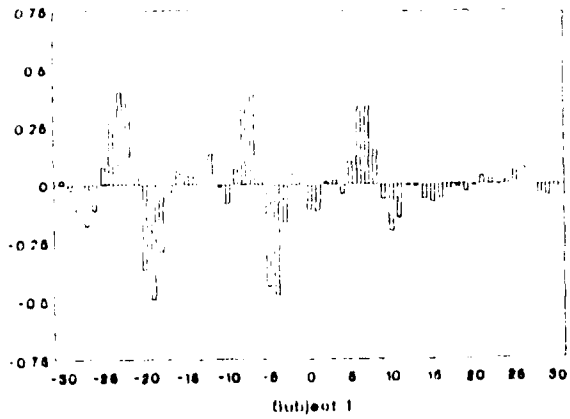
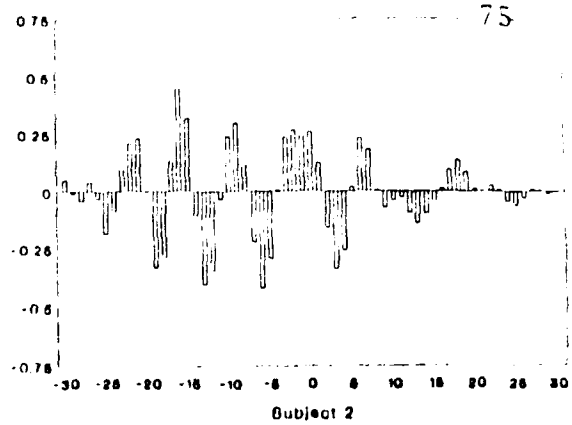
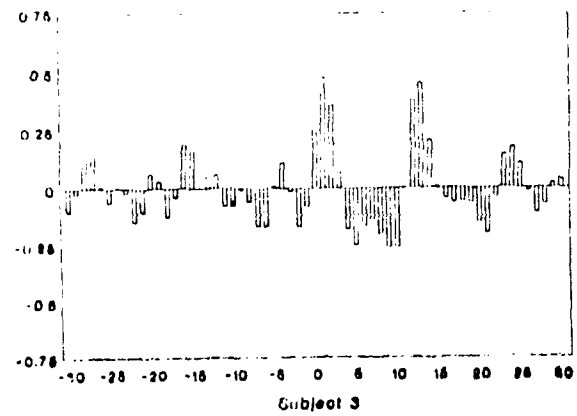
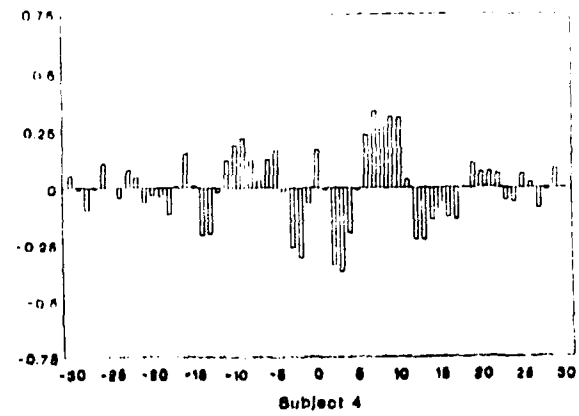


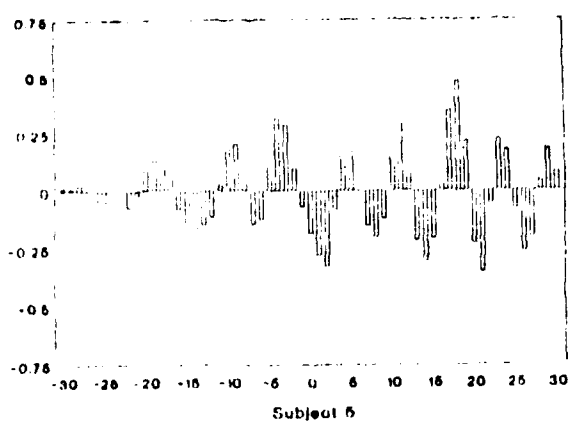
Figure 17 - RT/N1 Latency Cross-Correlation Functions

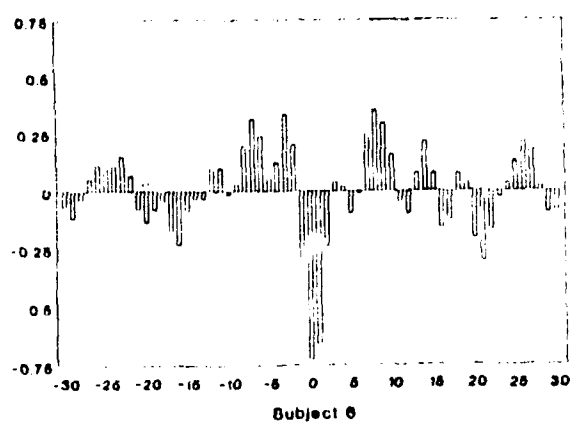


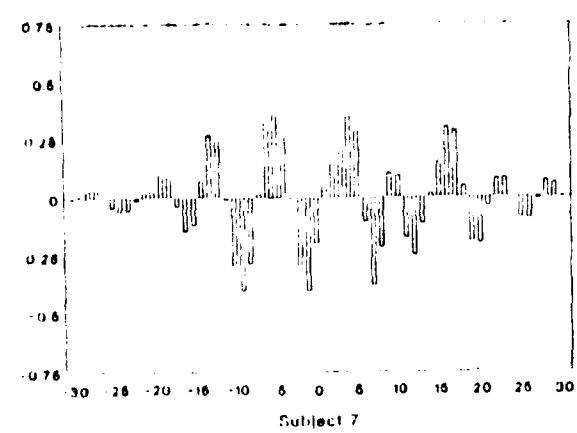












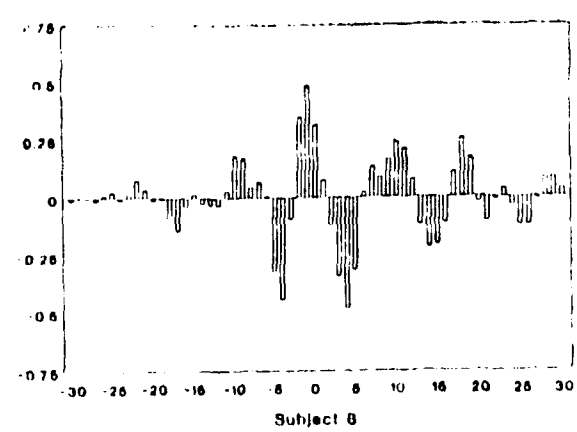


Figure 18 - RT/N1 Amplitude Cross-correlation Functions

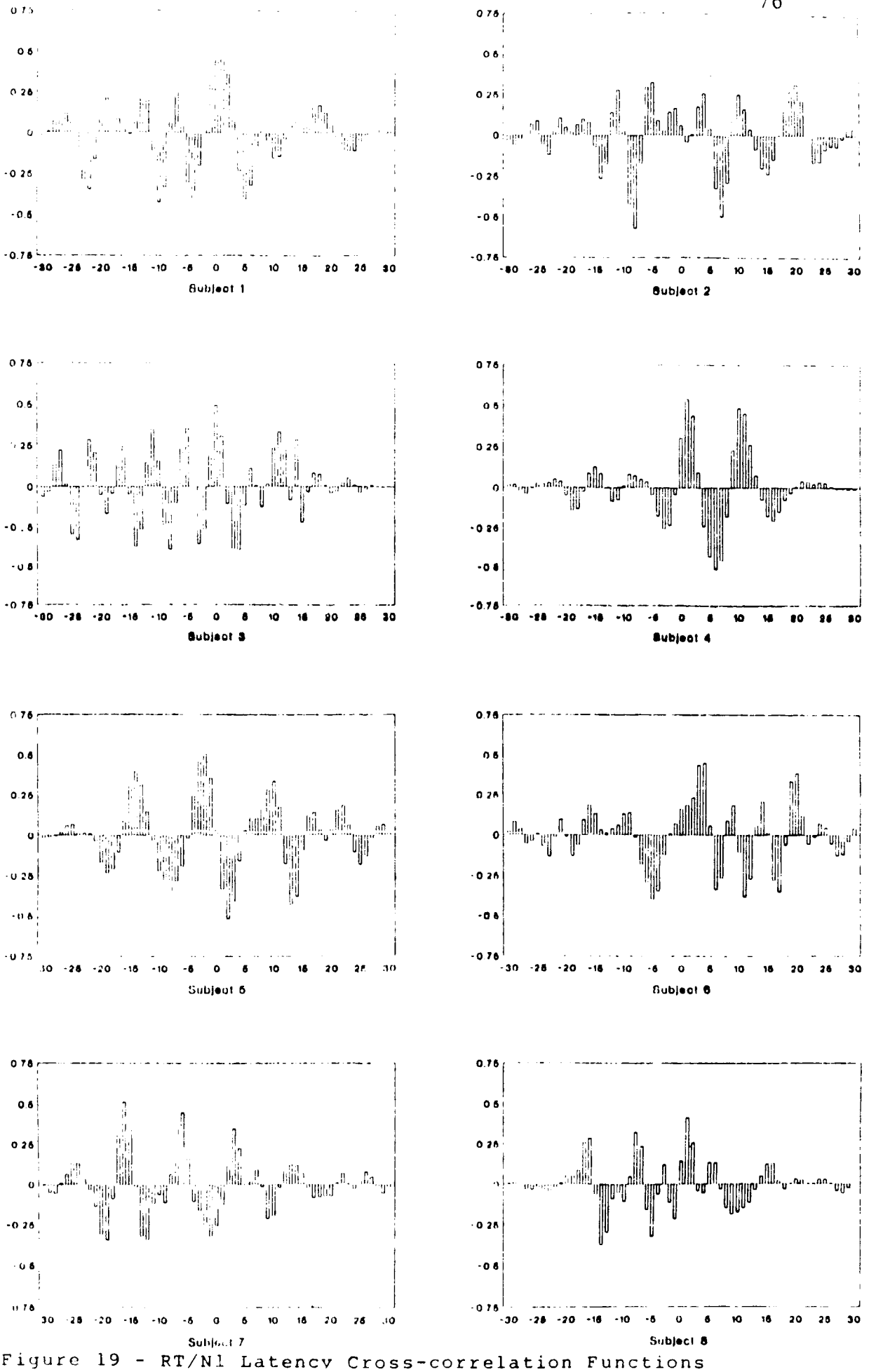


Figure 19 - RT/N1 Latency Cross-correlation Functions

RT/N2 AMPLITUDE

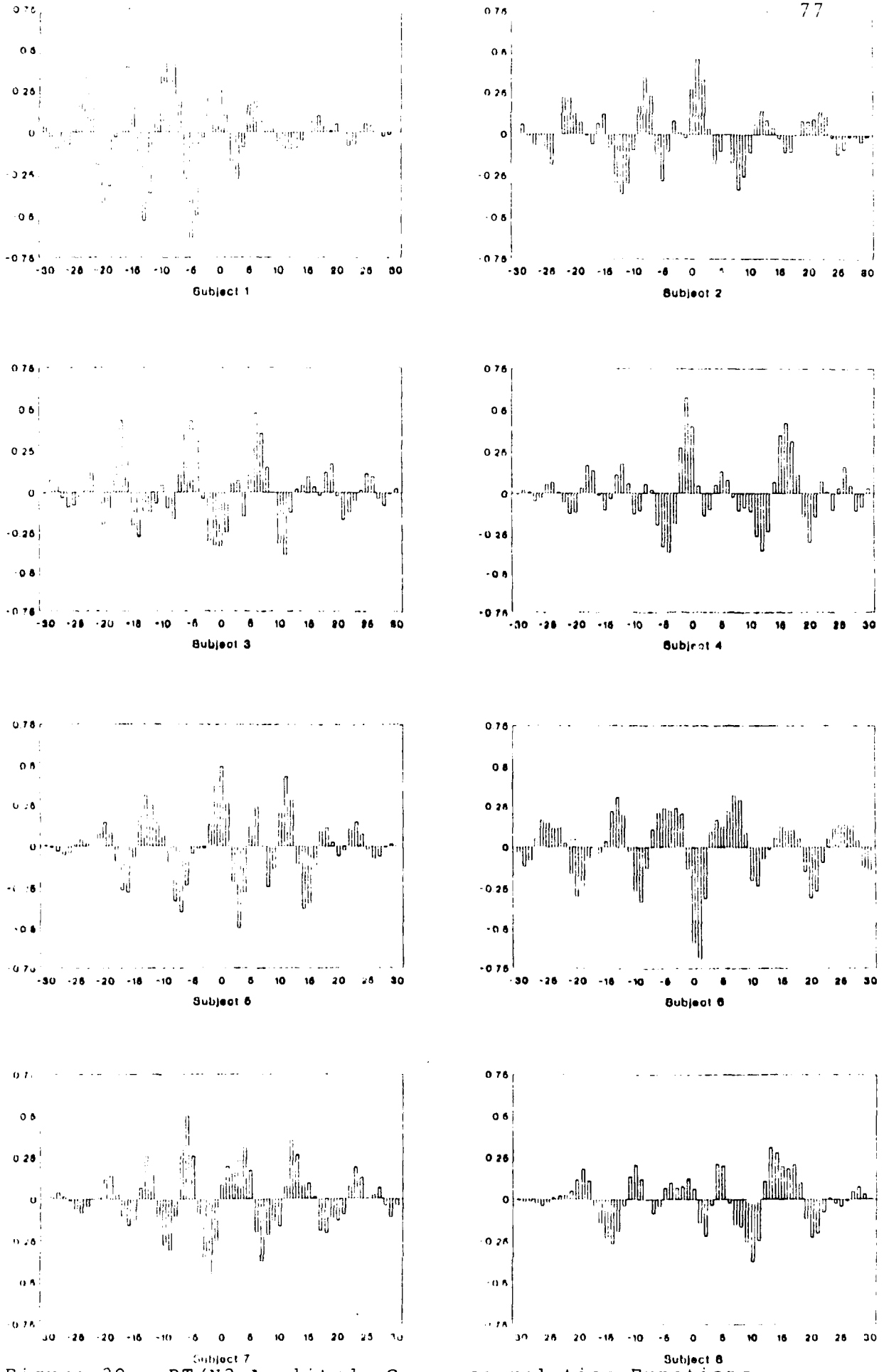


Figure 20 - RT/N2 Amplitude Cross-correlation Functions

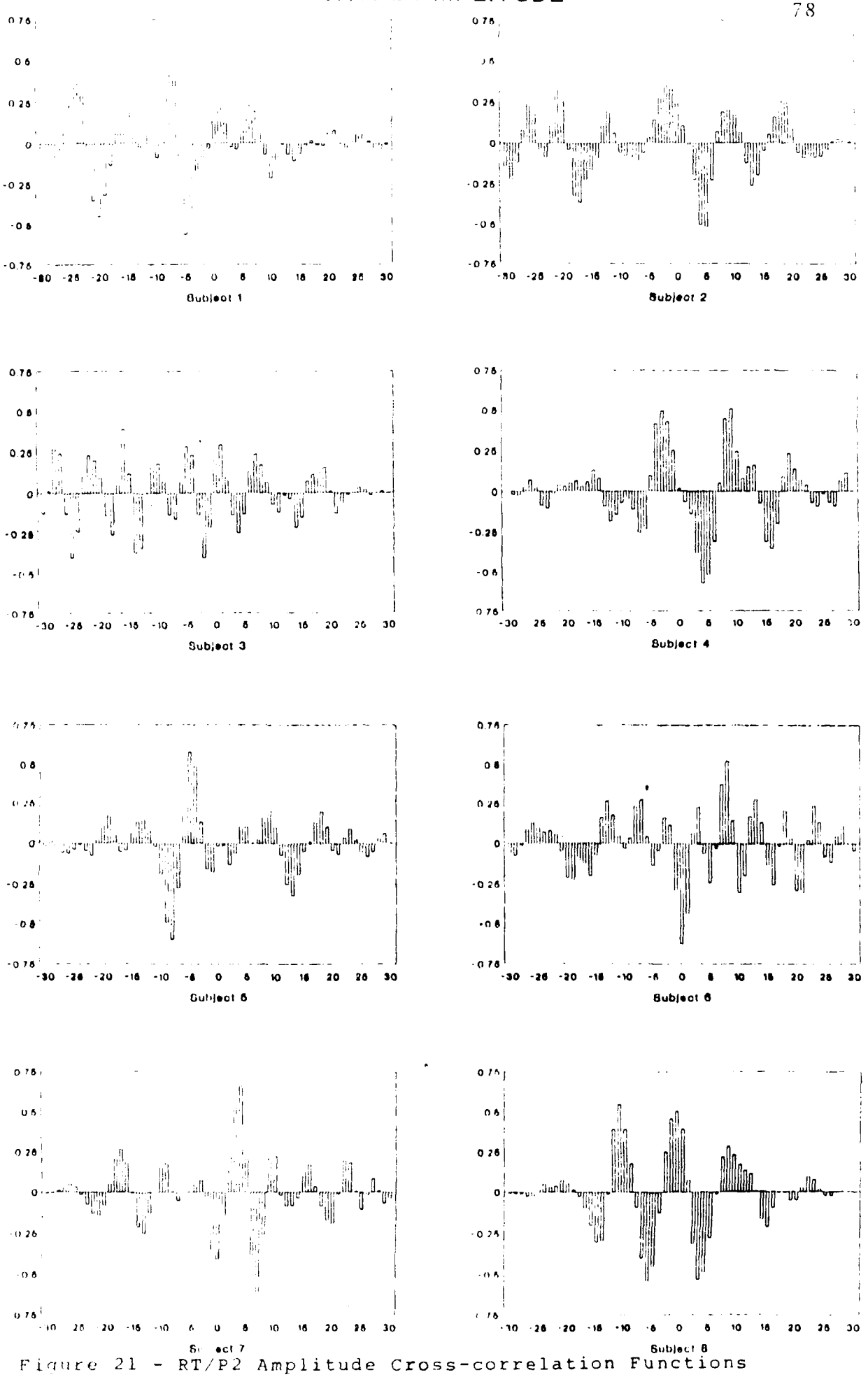


Figure 21 - RT/P2 Amplitude Cross-correlation Functions

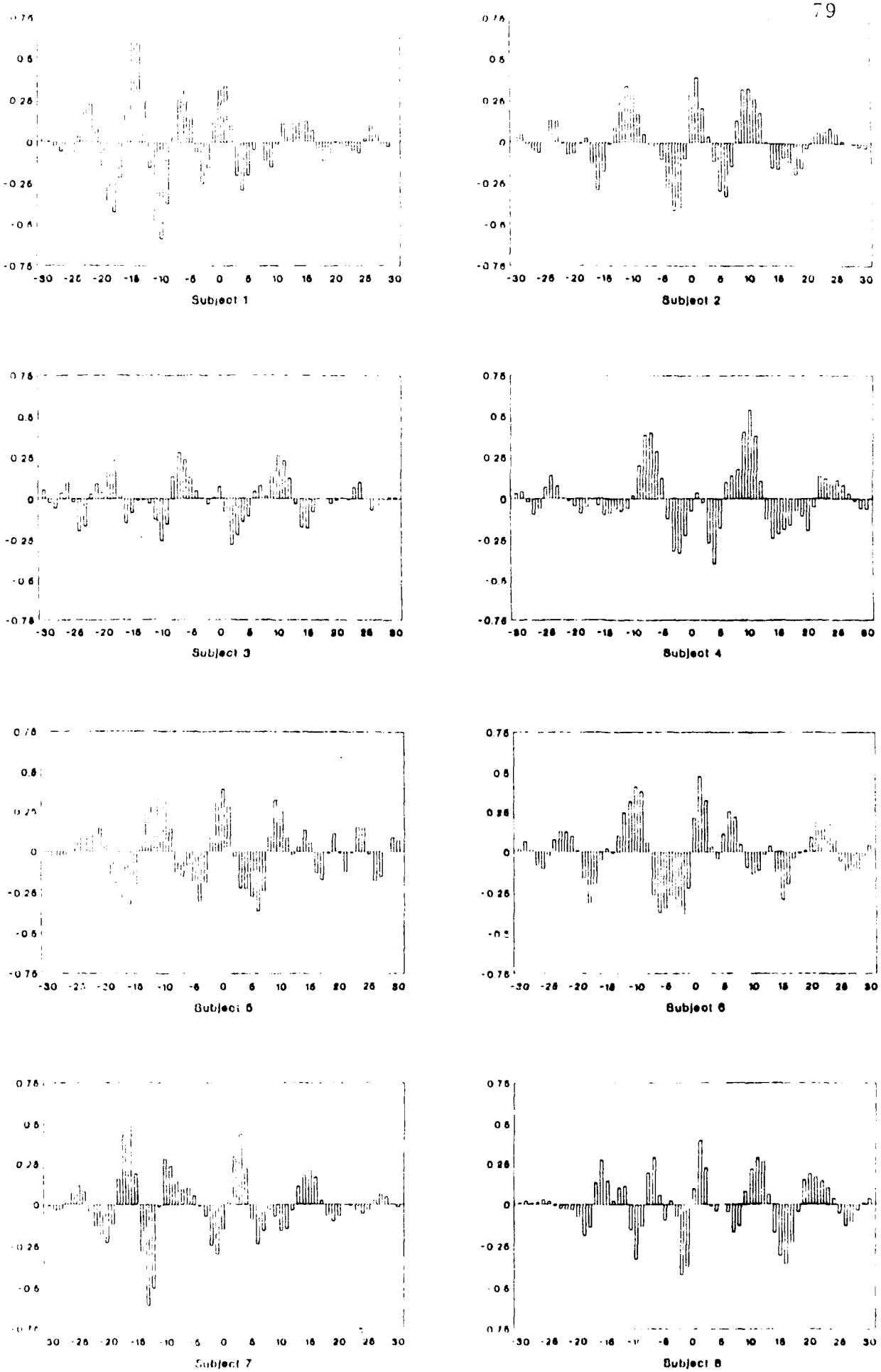


Figure 22 - RT/P3 Latency Cross-correlation Functions

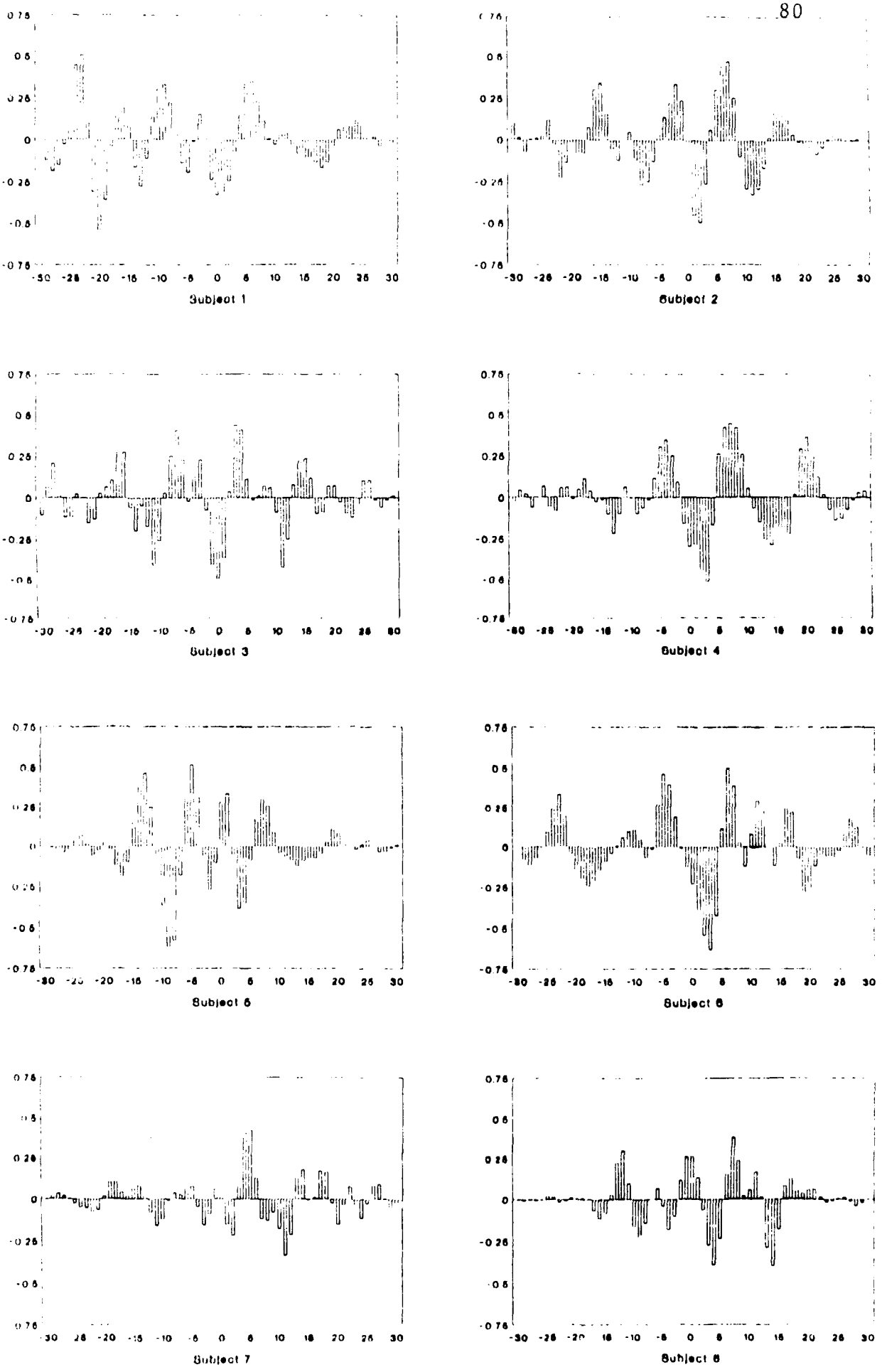


Figure 23 - RT/P3 Amplitude Cross-correlation Functions

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6.5 Experiment 5 - Repeated Testing, and Interblock Interval Effects on P300 Amplitude

Interest in the P300 since its discovery [24] has been considerable. Factors affecting amplitude and latency of the P300 include, among others, task relevance [22], stimulus probability [8,10], and stimulus value [1]. Such amplitude and latency effects suggest that P300 relates to changes in cognitive activities. The exact nature of these activities remains to be firmly established, but may include activities such as uncertainty resolution [24], context-updating [7], and resource allocation [14]. However, the list of experimental manipulations affecting P300 amplitude and latency continues to grow [13], suggesting that the pool of factors affecting P300 has not been exhausted.

One factor which has received only minimal attention when recording evoked potentials is time of day. Of those studies which have addressed time of day, conflicting results are reported. One study [3] presented evidence of higher P300 amplitude at 1000 hrs than at 1400 hrs for normal subjects. Furthermore, amplitude measures taken across the day (1000, 1200, 1400, 1600 and 1800 hrs) displayed a sinusoidal pattern of change. For an age, sex, education and IQ-matched group of untreated narcolepsy-cataplexy patients, however, the reverse relationship held: amplitudes were slightly higher at 1400 hrs than at 1000 hrs. Other findings conflict with the above regarding time of day variations in normal subjects. Another P300 study [15], using a signal-detection paradigm, reported that amplitude of the late positive component (LPC) displayed a diurnal variation; however, amplitude at 1000 hrs was significantly lower than amplitudes at 0400 hrs, 1600 hrs or 2200 hrs. This late positivity may have included other late positive slow-wave components as well as P300. Thus, it is still unclear whether a relationship between P300 and time of day exists.

Research addressing the effects of time of day (i.e. , "circadian rhythms") on behavioral measures has shown that psychomotor (speed) measures tend to peak in the afternoon while measures of short-term memory (e.g. , digit span, free recall) tend to show a morning superiority [2, 11]. The suggestion that behavioral measures peak at different times of day may have implications for research regarding cognitive factors indexed by evoked potentials. Given that P300 is thought to index higher cognitive capacities, it might be expected that a time of day variation in P300 would correspond with behavioral measures of the same underlying capacities.

A second variable which has recently drawn attention is the effect of repeated testing on amplitude of P300 to "target" stimuli [20]. That the P300 rapidly habituates to "nontarget" or "task-irrelevant" stimuli is well documented [18]. However, initial research led to the suggestion that P300 amplitude to targets was relatively resistant to change due to repeated testing [6]. Furthermore, most studies assessing "long-term"

habituation of the P300 are of relatively short duration, i.e., less than one hour for a complete experimental session [20].

Conflicting findings have been reported regarding repeated testing [19,20]. One study [20] assessed changes in P300 amplitude across a 3-hour experimental session and reported that P300 to both target and nontarget visual stimuli did not habituate. In contrast, another study [19] reported that P300 amplitude to correctly detected targets declined across successive 10-minute blocks of a 30-minute auditory vigilance task. Amplitude decreased from approximately 10 uV (first 10 minutes of vigilance) to approximately 5 uV (last 10 minutes of vigilance). Although details of the latter study were not reported, one methodological difference between these two studies was the length of the interblock interval. The latter study [19] tested continuously across 10-minute "blocks" (periods) of the vigilance task. In effect, no interblock interval was used. Conversely, the former study [20] allowed subjects a 2-minute rest interval between 10-minute blocks. This extended interblock interval may have resulted in dishabituation of P300 between blocks.

The present study addressed three questions. The first question addressed whether time of day factors affected P300 amplitude. A second question concerned whether amplitude of the P300 to target stimuli would show a decrement (habituate) with repeated testing, and whether this factor interacted with time of day. Our final question addressed whether the pattern of change in P300 amplitude due to repeated testing would be altered by extending the interblock interval between one pair of test blocks.

Method

Subjects Fifty normal, healthy undergraduate students (32 females, 18 males) enrolled in Psychology 201 courses participated in the experiment. All were screened for hearing, health/sleep problems, and medication use (e.g. , stimulants). They were told to obtain a normal night's sleep prior to participation and to refrain from alcohol and caffeine tne day of the experiment.

Apparatus A Grass Model 7P122 DC amplifier (time constant = .1 seconds, half-pass filter = 35 Hz, 60 Hz notch filter out) was used to record and filter the auditory evoked potential (AEP). Averaging was controlled by Computerscope Hardware/Software driven by an Apple IIe microcomputer. Tone stimuli (rise time = 4 ms) were controlled by a Commodore 64 microcomputer. Grass Model 7P211 AC amplifiers were used to record electroencephalographic (EEG), electrooculogram (EOG) and electromyogram (EMG) signals during daytime naps. All signals were recorded using Grass silver/silver chloride electrodes filled with Grass EC2 electrode cream.

Procedure Subjects were instructed to arrive at the Psychophysiology Laboratory at 0800 hrs for morning sessions or 1400 hrs for the afternoon sessions. They were given a brief description of the procedure upon arrival and an informed-consent

sheet to sign. Next, areas for EOG (outer canthi, above and below midline) and EMG (mental-submental) were cleaned, abraded, and electrodes applied with tape. Areas for EEG (Cz, Oz, left and right mastoids) were cleaned, abraded, and electrodes secured with collodion. Resistance was kept below 5 Kohm for all electrode sites. Subjects then were seated in a recliner and given instructions to count high-pitched (target) tones and to make a brief finger-lift response to each target tone. They then put on headphones and several low and high pitched tones were presented. Both low and high-pitched tones were 500 ms duration, 65 dB SPL against an ambient room background of 55 dB, and were presented binaurally through the headphones. Probability of the high-pitched target tones was .2 and the low-pitched nontarget tones was .8. Subjects were told to keep their eyes closed and to avoid sudden body movements during stimulus presentations. The experimenter monitored the subjects continuously during stimulus presentations to ensure that a finger-lift response occurred for every target presentation. Subjects who failed to respond on every occasion were eliminated from statistical analyses. Target and nontarget tones were presented in a random order with a fixed 1500 ms interval (tone onset to tone onset). The number of targets presented for each block varied from 35 to 45 so that subjects could not determine the number of tones presented without counting. The first 35 artifact-free targets were averaged. Evoked potentials contaminated by excessive eye or muscle artifact were rejected on-line by the Computerscope. The EEG was sampled from the Cz placement for 100 ms prior to stimulus onset and for an additional 710 ms following stimulus onset at a rate of one sample every 3.96 ms. At the end of each block, subjects reported how many target tones they counted.

From each of the morning (n = 25) and afternoon (n = 25) groups, 10 subjects were randomly assigned to a one-hour "awake" condition and 10 were assigned to a one-hour "nap" condition (see Footnote 1). The remaining 10 subjects (5 morning, 5 afternoon) were assigned to a "control" condition. All subjects (n = 50) were tested across six blocks. The interval (interblock interval) between Blocks 1 and 2 and between Blocks 3, 4, 5 and 6 was 30 seconds for all conditions (awake, nap and control). However, the interval between Blocks 2 and 3 was different for the awake and nap conditions versus the control condition. The interval between Blocks 2 and 3 was one hour for subjects in the awake and nap conditions (subjects in the latter condition were allowed to nap during this time) and 30 seconds for subjects in the control ("equal interblock interval") condition.

Results Amplitude of P300 was measured as the difference between baseline averaged over the 100 ms prestimulus period and peak of the most positive-going component occurring approximately 300 ms subsequent to stimulus onset (mean P300 latency = 327.98 ms). Figure 1 illustrates P300 amplitude across test Blocks 1 - 6 for morning versus afternoon groups (collapsed across Nap, Awake, and Control conditions). As seen in Figure 1, amplitude of P300 demonstrated a marked time of day effect. Overall

amplitudes were higher in the morning compared to afternoon across all blocks. The difference between morning and afternoon sessions was confirmed statistically (Time of Day main effect, $F[1, 44] = 6.00$, $p < .05$). Although the pattern of amplitude decrement across blocks appeared to differ for morning versus afternoon sessions, the Time of Day x Block interaction was not significant.

Repeated testing Figure 2 displays P300 amplitude across blocks for subjects in the awake, nap, and control conditions (collapsed across Time of Day). As evident from Figure 2, amplitude of P300 decreased slightly from Block 1 to 2 for the awake and nap conditions but increased slightly for the control condition. Amplitude increased from Block 2 to 3 for both the awake and nap conditions (one-hour interval) but showed a sharp decrease for the control condition (30-second interval). Amplitude decreased across the next three blocks for all three conditions. The decrease from Block 3 to 4 appeared to be greater for the awake and nap conditions than for the control condition. A graphic illustration of the decrement in P300 amplitude across the experimental session can be seen in Figure 3. This figure represents the averaged waveform for Block 1 (mean) vs. Block 6 (mean), collapsed across Time of Day and Condition factors. As seen in Figure 2, a decrement in P300 amplitude occurred from Block 1 to Block 6. Also evident fror Figure 2 is that the decrement in P300 was due to a diminution in the overall area of P300 as opposed to a redistribution of the area of the curve [16]. A significant Block main effect was found ($F[5, 220] = 18.94$, $p < .05$). Post-hoc Tukey HSD performed on mean P300 amplitude for Blocks 1 through 6 revealed that amplitude at Block 6 was lower than Blocks 1 through 4 ($q[6, 220] = 1.69$) and amplitude at Blocks 4 and 5 were lower than Blocks 1, 2 and 3. Blocks 1, 2, and 3 did not differ nor did Blocks 4 and 5 or Blocks 5 and 6.

Interblock interval As noted above and in Figure 2, the awake and nap conditions demonstrated an increase in P300 amplitude across the extended interval from Block 2 to 3. The increase from Block 2 to 3 was higher for the awake condition (mean increase of 1.09 uV) than for the nap condition (mean increase of .49 uV). The control condition (30-second interval), however, demonstrated a marked decrease in amplitude ($x = 3.73$ uV) from Block 2 to 3. An ANOVA performed on P300 amplitude (Blocks 2 to 3 only) revealed a significant Condition x Block interaction, $F[2, 44] = 5.31$, $p < .05$), confirming these observations. Further one-way analyses comparing P300 amplitude from Block 2 to 3 separately for each condition revealed that while the amplitude decrement from Block 2 to 3 was significant for the control group, $F(1, 9) = 8.95$, $p < .05$, the amplitude increase from Block 2 to 3 for the nap and awake groups was not significant ($p > .05$). No further main effects or interactions were significant.

Discussion Amplitude of P300 clearly was affected by time of day. These effects are consistent with prior research [3]

assessing amplitude changes in normal subjects across the day (i.e., higher P300 amplitudes in the morning than in the afternoon). That time of day impacts on P300 amplitude is important in that it may reflect cognitive processes whose behavioral measures vary in the same direction. This time of day effect for P300 amplitude in fact parallels variations in behavioral tasks involving memory capacities such as speed of learning noted as early as 1885 by Ebbinghaus [26], and more recently for tasks of immediate memory such as digit span [2] and of short-term memory such as free P300 recall [11], i.e. , better performance in the morning than in the afternoon. Moreover, the decrement in P300 amplitude noted for the afternoon condition may coincide with the decrement noted for behavioral measures. That is, the first test block of the afternoon condition in the present study (approximately 1430 hrs) occurred within a time frame previously demonstrated to correspond with a temporary decrease in performance [2] between 1300 and 1500 hrs [2,4]. Thus, it is possible that changes in both performance [2] and P300 amplitude [3, present study] reflect changes in cognitive capacities. Although only two test points were assessed within a 24-hour period in the present study, the direction of P300 amplitude change is consistent with others [3] and warrants further investigation of variations in P300 amplitude across 24 hours.

Repeated Testing and Interblock Interval Effects Amplitude of P300 clearly showed habituation with repeated testing. The rate of habituation, however, was altered by inserting a one-hour interval between blocks. Whether the interval consisted of sleep or wakefulness was not an important factor, as both wake and nap conditions demonstrated a slight increase in amplitude from Block 2 to 3. Thus, P300 amplitude is affected by both repeated testing and the interblock interval. The P300 habituation across blocks noted in the present study may have been related to the interstimulus interval. Others have demonstrated that interstimulus intervals affect P300 amplitude. For example, prior research [10] has demonstrated that P300 amplitude was lower at shorter interstimulus intervals (higher temporal probability), regardless of sequential probability. However, that study [10] did not determine the effects of interstimulus intervals across repeated testing (i.e. , habituation). The present results suggest that any effects due to short interstimulus intervals [10] can be attenuated by the length of the interblock interval. In effect, inserting a long interblock interval eliminated habituation on the subsequent block. Another study [20] also reported no decrement in P300 amplitude to target stimuli with long (2 minutes) interblock intervals. Thus, both interstimulus and interblock intervals can affect the P300 component.

The finding that P300 amplitude decreases with repeated testing also may relate to the allocation of resources for the detection and counting task [12]. That is, due to perceived experimental demands, etc., subjects may have "over-allocated"

resources to the task during the initial test blocks. This allocation is reflected in high amplitude P300. However, because the task is relatively easy, with continued testing subjects learn to allocate fewer resources to the task (P300 decreases) while continuing to perform accurately. Although the present study did not directly assess "resource allocation" by invoking a concurrent task [12,23], subjects did report engaging in other mental activities (e.g. , daydreaming) while performing the task. Others have reported that P300 amplitude to counted tones decreases when the counting task is made secondary although counting accuracies themselves are not affected significantly [12]. It is unlikely that methodological factors contributed to P300 habituation. One might argue that the use of a short time constant (.1 seconds) affected P300 amplitude. In effect, P300 amplitude is lower at shorter time constants [9]. However, since the time constant was not altered across blocks, any effect of attenuation on P300 amplitude would be constant across all blocks. More important, P300 habituation with repeated testing also has been demonstrated using a longer (.8 seconds) time constant [16] and the difference in P300 amplitude between a time constant of .8 seconds and much longer ones (e.g., 10 seconds) is minimal. Second, the long stimulus duration (500 ms) may have affected P300. However, P300 amplitude decrements have been replicated both at this stimulus duration [16] and at shorter (50 ms) stimulus durations [17]. Finally, although changes in P300 component structure was not assessed via multiple site (scalp distribution) measures, similar P300 habituation effects with repeated testing have been recorded at both the parietal (Pz) and frontal (Fz) scale sites [16].

In sum, the present study indicates that the processes indexed by P300 are affected by time of day, repeated testing, and interblock intervals. Time of day effects suggest a circadian variation in cognitive processes underlying P300, while repeated testing and interblock interval effects suggest changes in the allocation of these processes with time on task.

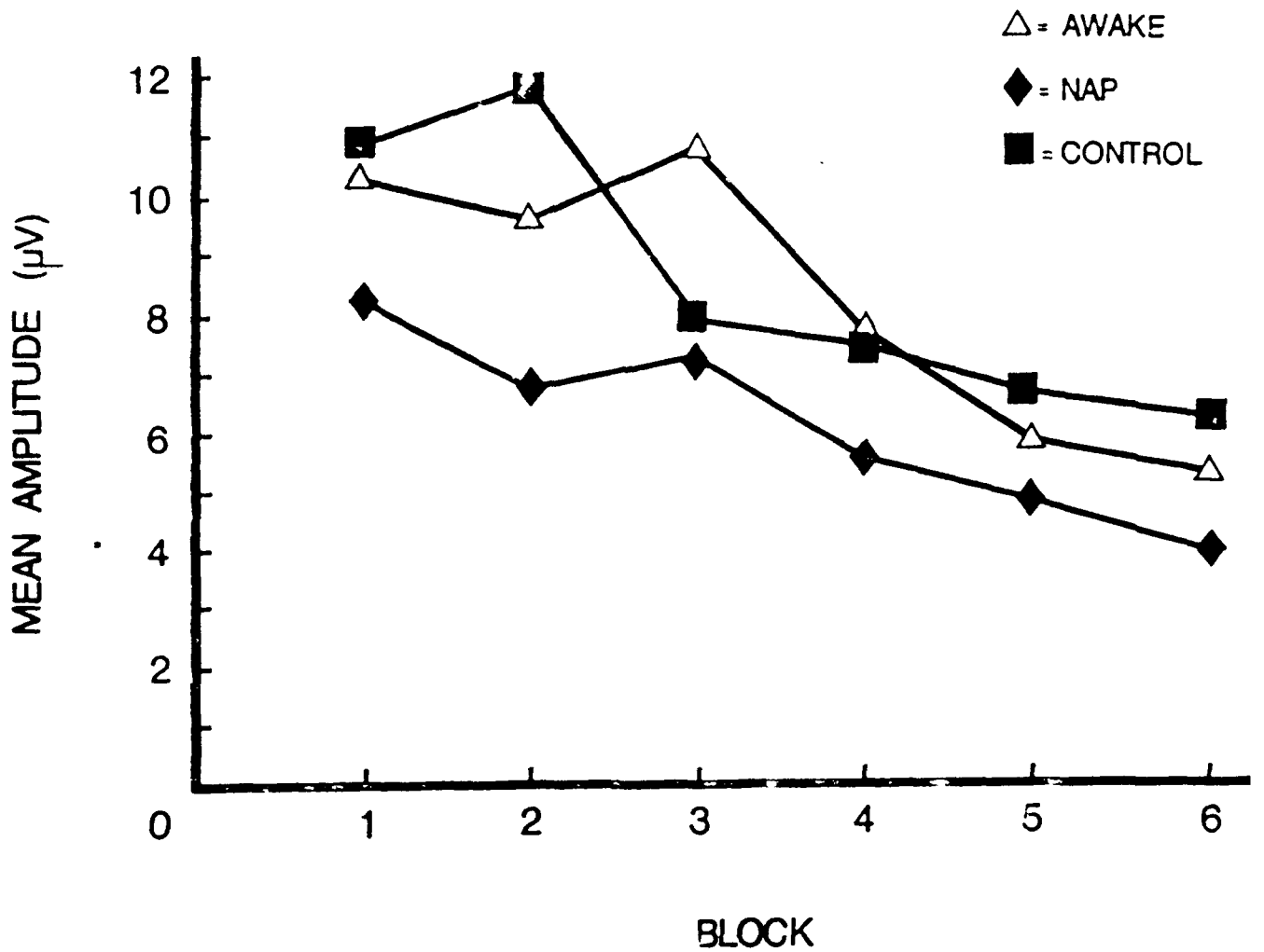

Figure 1. P300 amplitude across blocks for morning versus afternoon sessions.

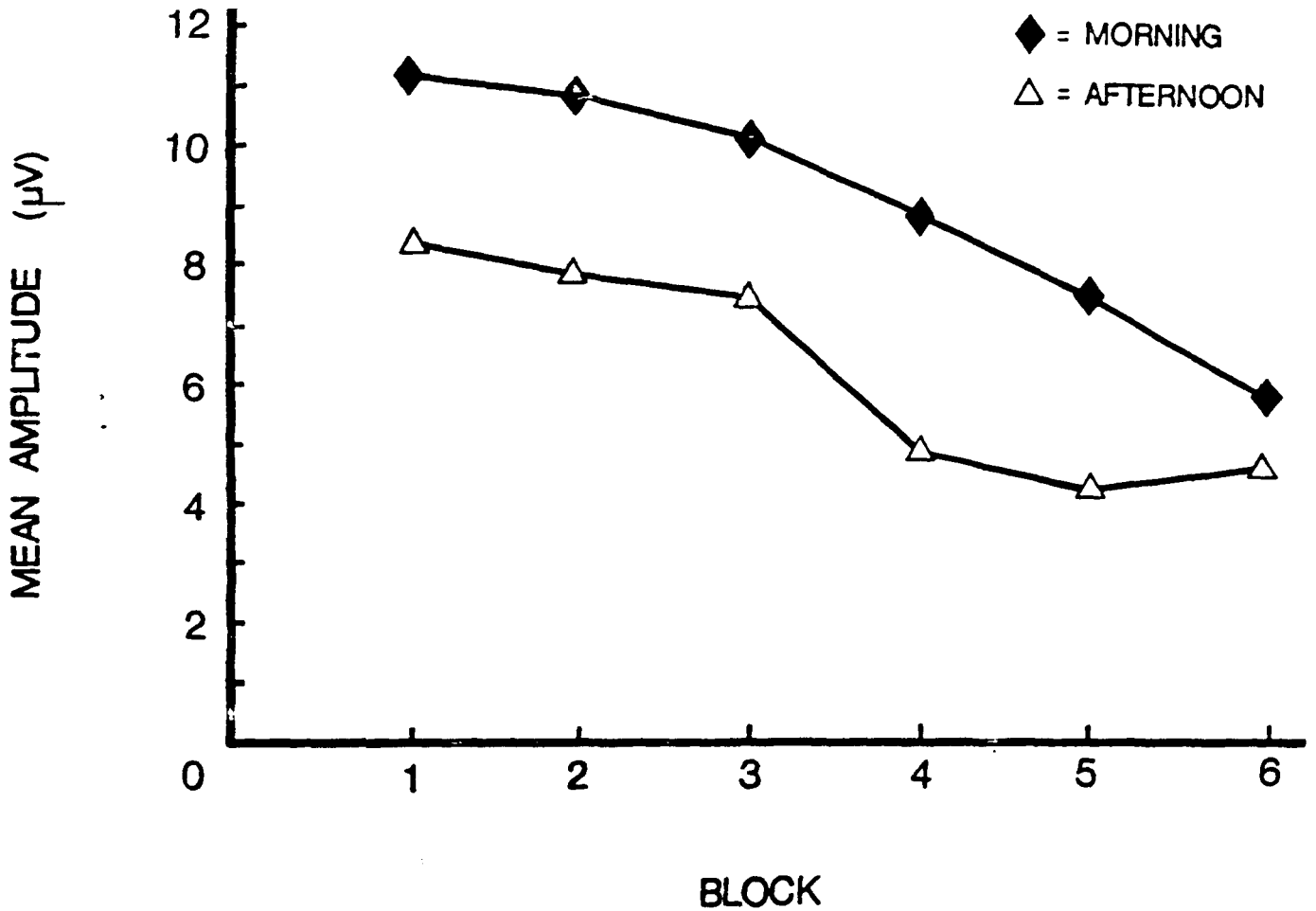

Figure 2. P300 amplitude across blocks for awake, nap and control conditions.

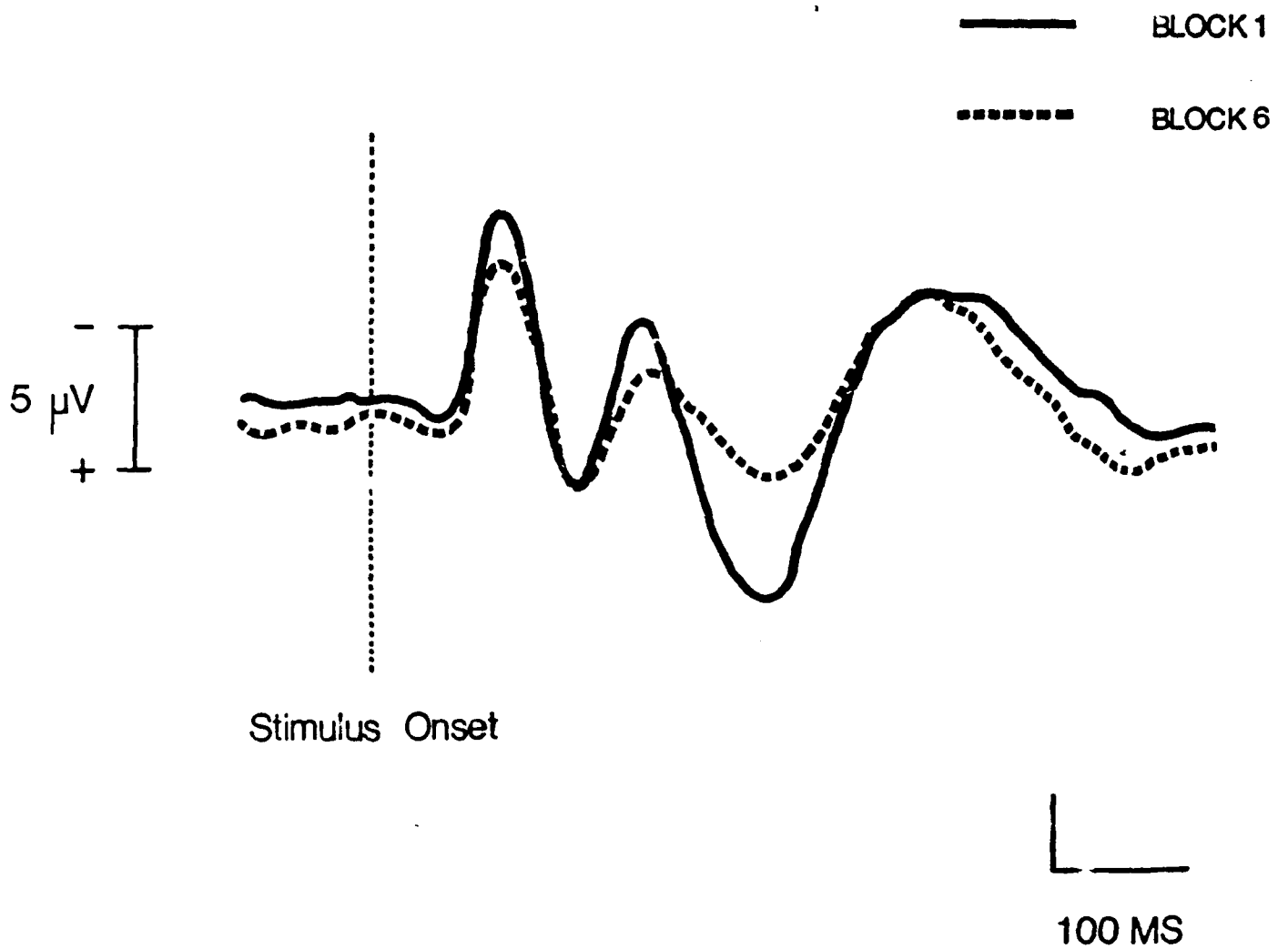

Figure 3. Averaged mean waveform for Block 1 versus Block 6.

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6.6 Experiment 6 - ERPs during the Wake/Sleep Transition

The questions addressed in this experiment were 1) What are the ERP changes associated with the Wake/Sleep transition? 2) Which of the ERP changes are associated with the decline in performance associated with the Wake/Sleep transition? and 3) What do the ERP changes tell us about changes in information processing during the Wake/Sleep transition?

During sleep, stimuli that normally elicit directed movement may no longer do so. Even those stimuli that signal the availability of goods or the presence of danger may not result in observable action. Reduced responsiveness is also associated with sleepiness. "Sleepiness" is a term often used in reference to the shortened sleep onset latencies associated with sleep loss, irregular sleep, and circadian factors.

Although progress has been made in describing decrements in responding related to sleep and sleepiness, little is known about the underlying processes. Sensory thresholds may be elevated, the capacity for information processing may be diminished, and/or the ability to select and organize responses may be depressed.

The present research concerns whether event-related brain potentials (ERPs) might provide useful information about the changes in responsiveness with sleep and sleepiness. The ERPs described here are scalp-recorded electrical potentials resulting from task-related stimulus presentation. ERPs reflect both psychological and physical properties of stimuli.

The research was motivated by the possibility that ERPs provide a reliable and unobtrusive index of information processing during sleep and sleepiness and might ultimately be useful in the assessment of performance readiness.

Method

Eight experimental subjects were scheduled for an afternoon test after having slept 3 hrs less than normal the night before. During the test, subjects performed an oddball task.

Oddball Task This task involved the random alternation of brief (.45 s) 1000 Hz and 1500 Hz binaurally-presented tones spaced 2 s apart. One tone was designated the target (p = .2). The other was designated the nontarget (p = .8). Subjects were instructed to respond to the target by a finger lift response and to ignore the nontarget. ERPs resulting from both target and nontarget presentations were recorded from three scalp locations (Fz, Cz, and Pz).

Wake/Sleep Test After being tested while awake and sitting in a chair, subjects were told that tones would continue to be delivered throughout the sleep period and they were to continue to respond to the target tone. Subjects were tested under these conditions until they had been in stage 2 sleep for 10 continuous minutes.

Control Subjects Four control subjects were tested following the above protocol except they were not given instructions to attend to the tones.

Sleep stages ERPs were averaged for stimuli presented

during wakefulness, stage 1A [fragmented alpha], stage 1b [stage 1], stage 2a [first 5 min of stage 2] and stage 2b [first 5 min of stage 2 preceded by 5 continuous min of stage 2 sleep].

Results

An ERP from an awake sitting subjects is shown in Figure 1. Grand average ERPs at each lead for targets and nontargets and for each sleep stage are shown in Figure 2. Latency and amplitude values for the ERPs obtained from experimental subjects are presented in Figures 3-5. Figure 6 presents control group data and Figure 7 shows the relationship between ERPs and responding. There are several statistically-reliable and important features of these data:

1) During wakefulness, P3 is larger for the target at the Pz electrode replicating much previous work;
2) N1 amplitude diminishes and P2 amplitude increases with sleep onset;
3) a prominent frontal negativity achieves maximum amplitude at stage 1B, then diminishes with sleep onset;
4) very pronounced changes in ERPs from wakefulness to sleep include a reduction in P3 amplitude, increased N2 amplitude, and increased N2 latency;
5) differences in target and nontarget ERPs during stage 2 include a larger target N2 amplitude and also a late centrally-dominant target positivity (800-1000 ms);
6) target ERPs for the control subjects more closely resemble nontarget ERPs; and
7) behavioral responsiveness (both likelihood and latency of responding) was correlated with N2 and P3.

Discussion

Previous studies have shown wake/sleep differences in ERPs to nonmeaningful stimuli. The present findings establish that task-relevance is an important determinant of ERPs even after 10 min or more of stage 2 sleep.

In research with alert subjects, N2 has been related to stimulus detection and P3 has been related to stimulus evaluation and classification. From this perspective, the findings of the present study suggest that sleepiness and sleep onset are associated with increases in the time required for stimulus detection, evaluation, and classification and that performance decrements are due at least in part to the changes in these processes.

The finding that changes in N2 and P3 were correlated with changes in responsiveness suggests that sleep ERPs reflect the same cognitive processes as wake ERPs (cf. Sams et al., 1983; Wesensten & Badia, 1988). However, the present study does not clearly establish that the ERPs recorded in sleep and sleepiness can be interpreted in the same manner. Further research is needed to evaluate the comparability of the determinants of wake and sleep ERPs.

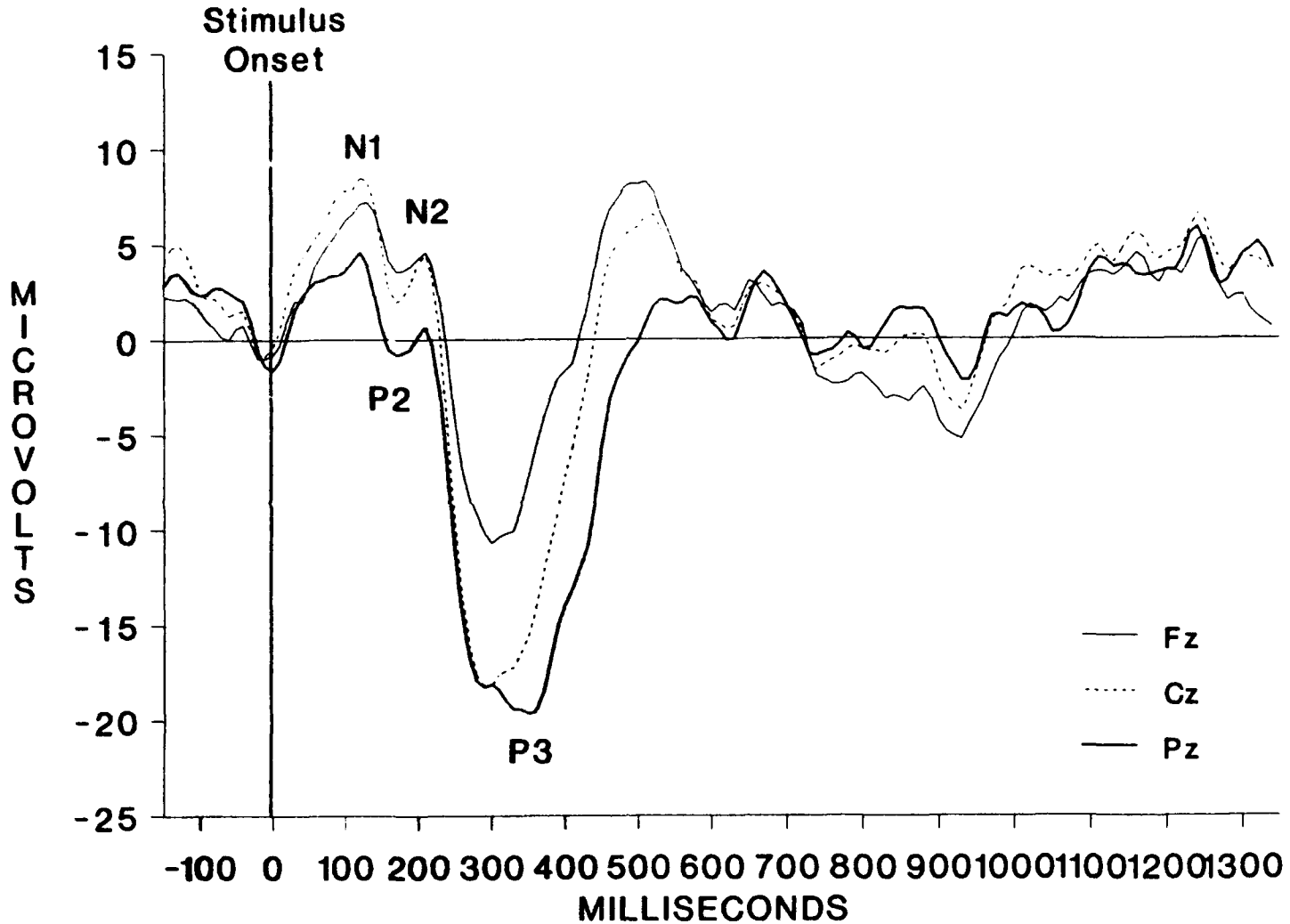


Figure 1 - An ERP from an awake sitting subject

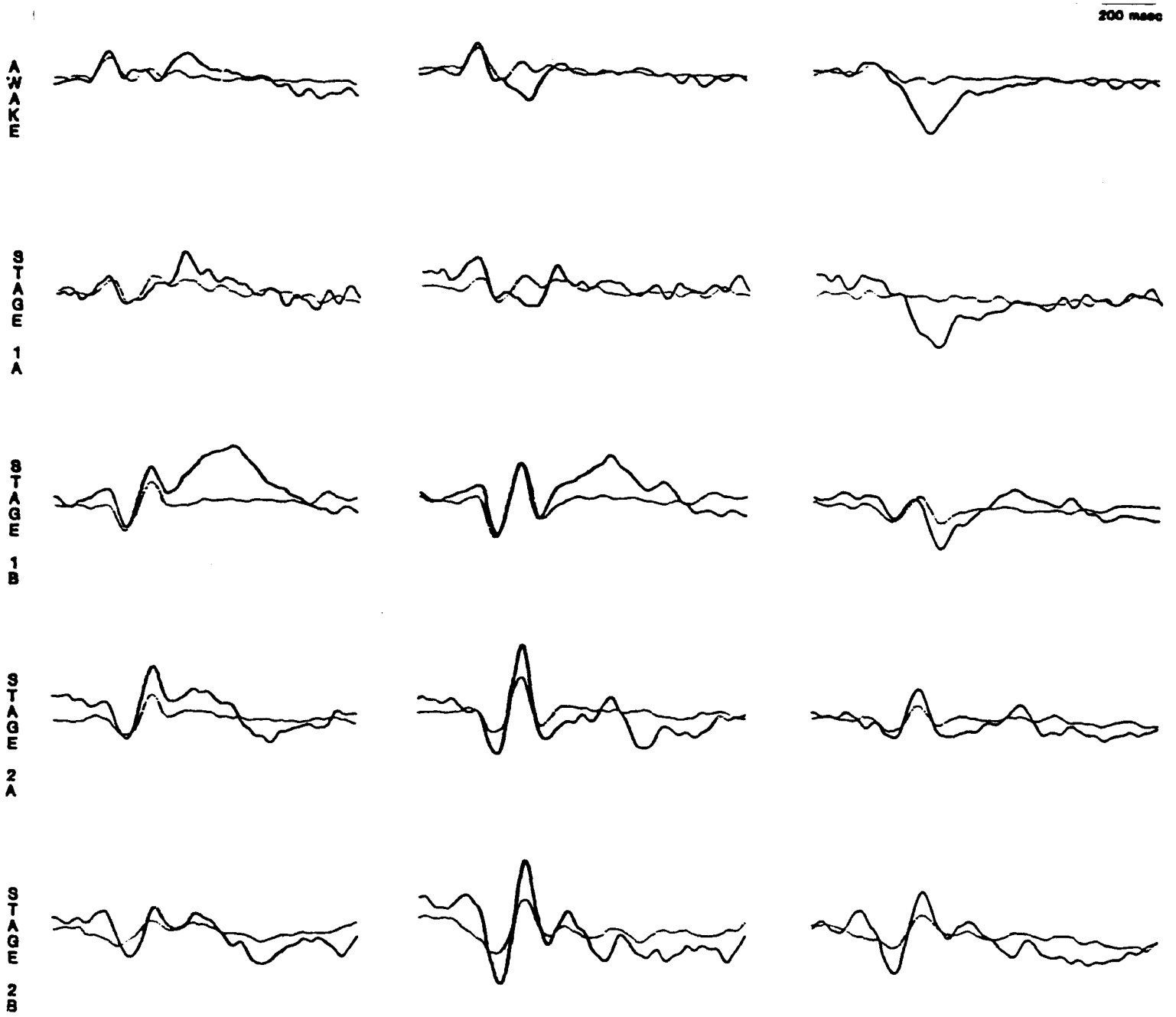
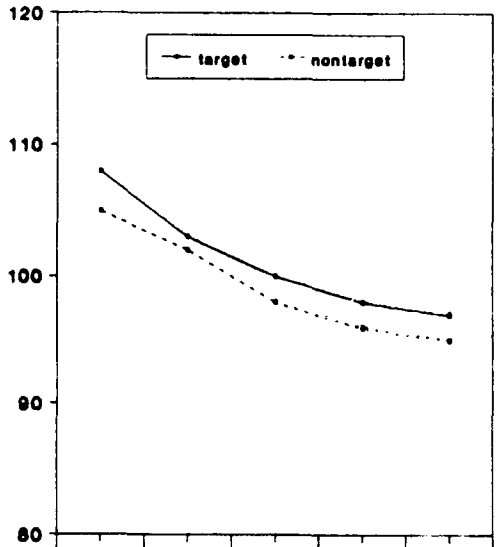


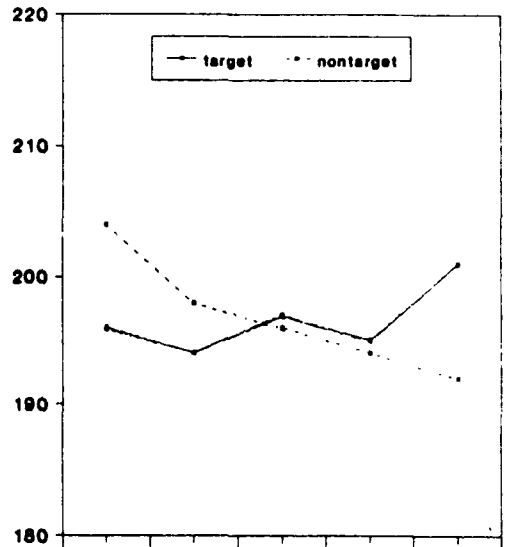
Figure 2 - Grand average ERPs for Experimental subjects

N1 Latency (ms)



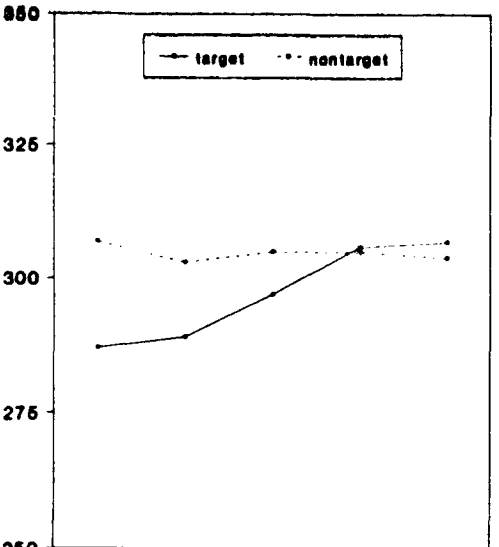
	Awake	1A	1B	2A	2B
target	108	103	100	98	97
nontarget	106	102	98	96	95

P2 Latency (ms)



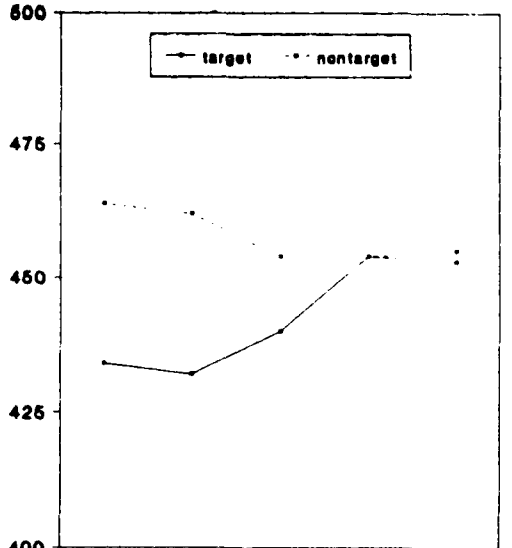
	Awake	1A	1B	2A	2B
target	196	194	197	195	201
nontarget	204	198	196	194	192

N2 Latency (ms)



	Awake	1A	1B	2A	2B
target	287	289	297	306	307
nontarget	307	303	305	305	304

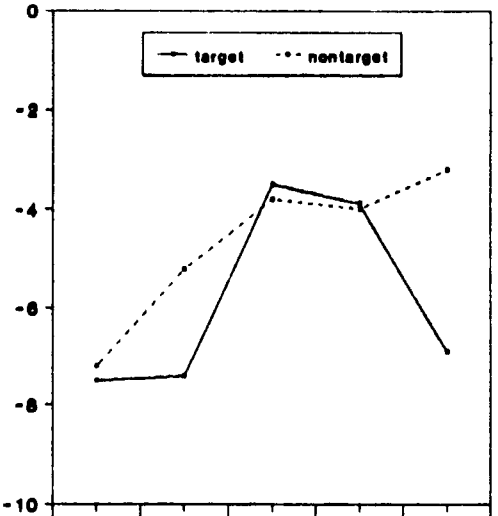
P3 Latency (ms)



	Awake	1A	1B	2A	2B
target	434	432	440	454	453
nontarget	464	462	454	454	455

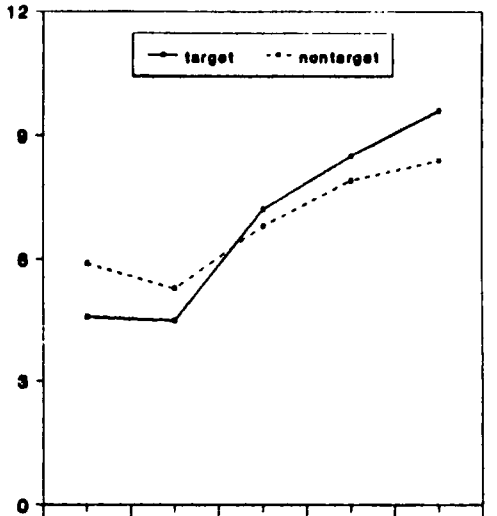
Figure 3 - Latency measures for N1, P2, N2, & P3

N1 Amplitude
in microvolts



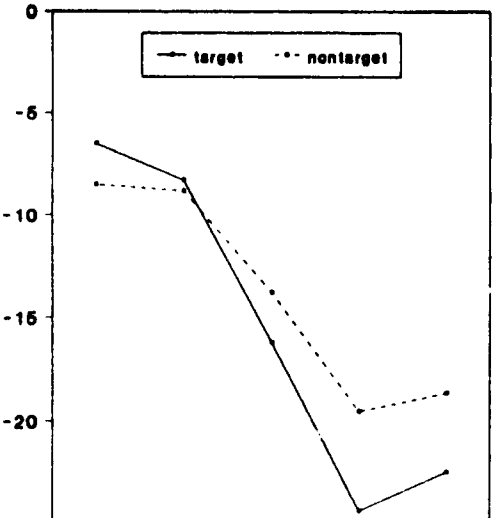
	Awake	1A	1B	2A	2B
target	-7.5	-7.4	-3.5	-3.9	-6.9
nontarget	-7.2	-5.2	-3.8	-4	-3.2

P2 Amplitude
in microvolts



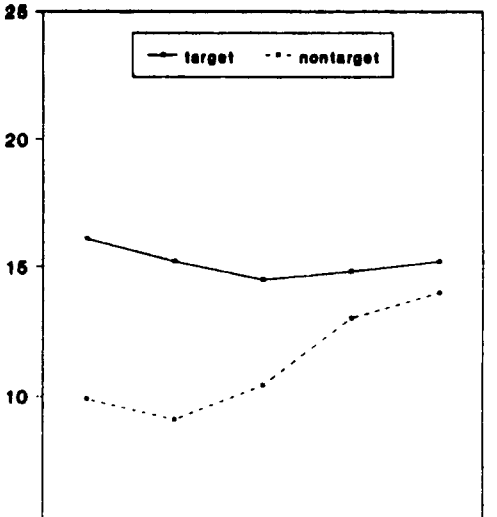
	Awake	1A	1B	2A	2B
target	4.8	4.6	7.2	8.5	9.6
nontarget	5.9	5.3	6.8	7.9	8.4

N2 Amplitude
in microvolts



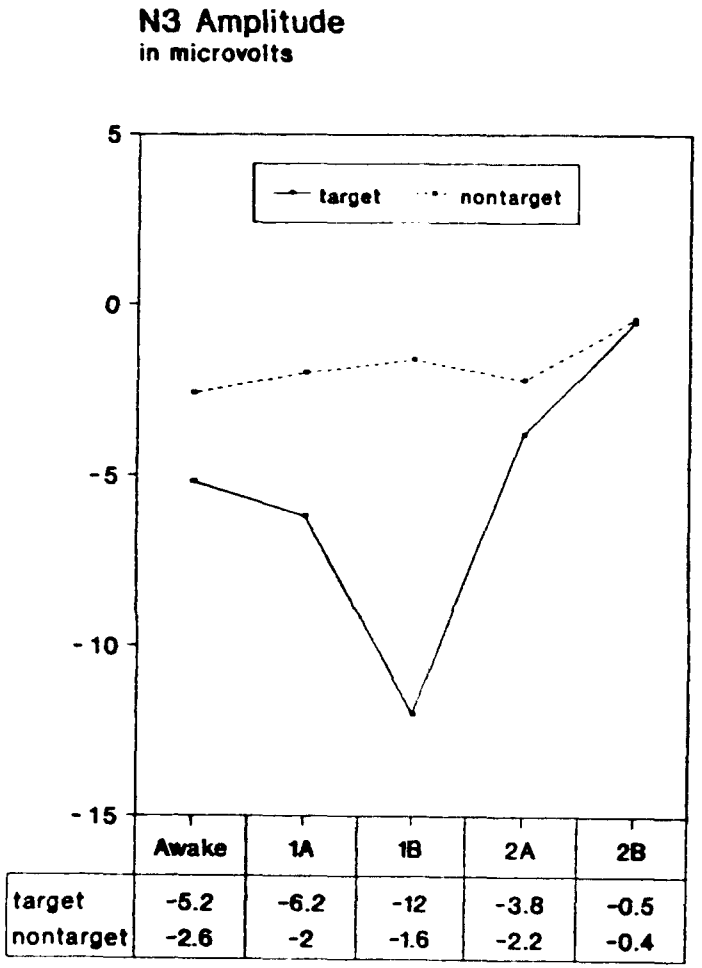
	Awake	1A	1B	2A	2B
target	-6.5	-8.3	-16.2	-24.3	-22.4
nontarget	-6.5	-6.8	-13.8	-19.5	-18.6

P3 Amplitude
in microvolts

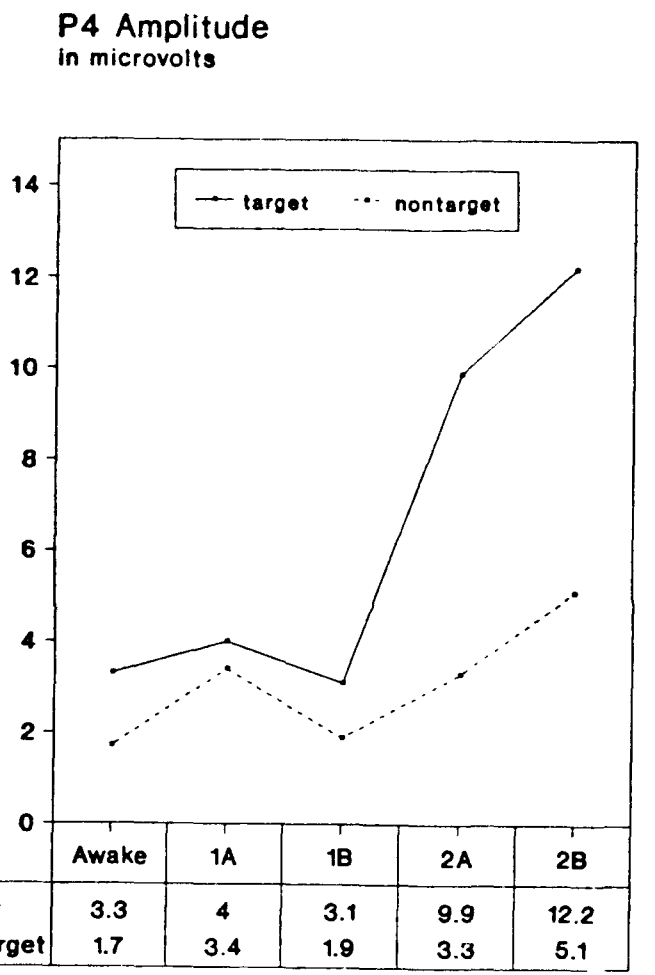


	Awake	1A	1B	2A	2B
target	16.1	15.2	14.5	14.8	15.2
nontarget	9.9	9.1	10.4	13	14

Figure 4 - Amplitude measures for N1, P2, N2, & P3



	Awake	1A	1B	2A	2B
target	-5.2	-6.2	-12	-3.8	-0.5
nontarget	-2.6	-2	-1.6	-2.2	-0.4



	Awake	1A	1B	2A	2B
target	3.3	4	3.1	9.9	12.2
nontarget	1.7	3.4	1.9	3.3	5.1

Figure 5 - Amplitude measures for N3 & P4

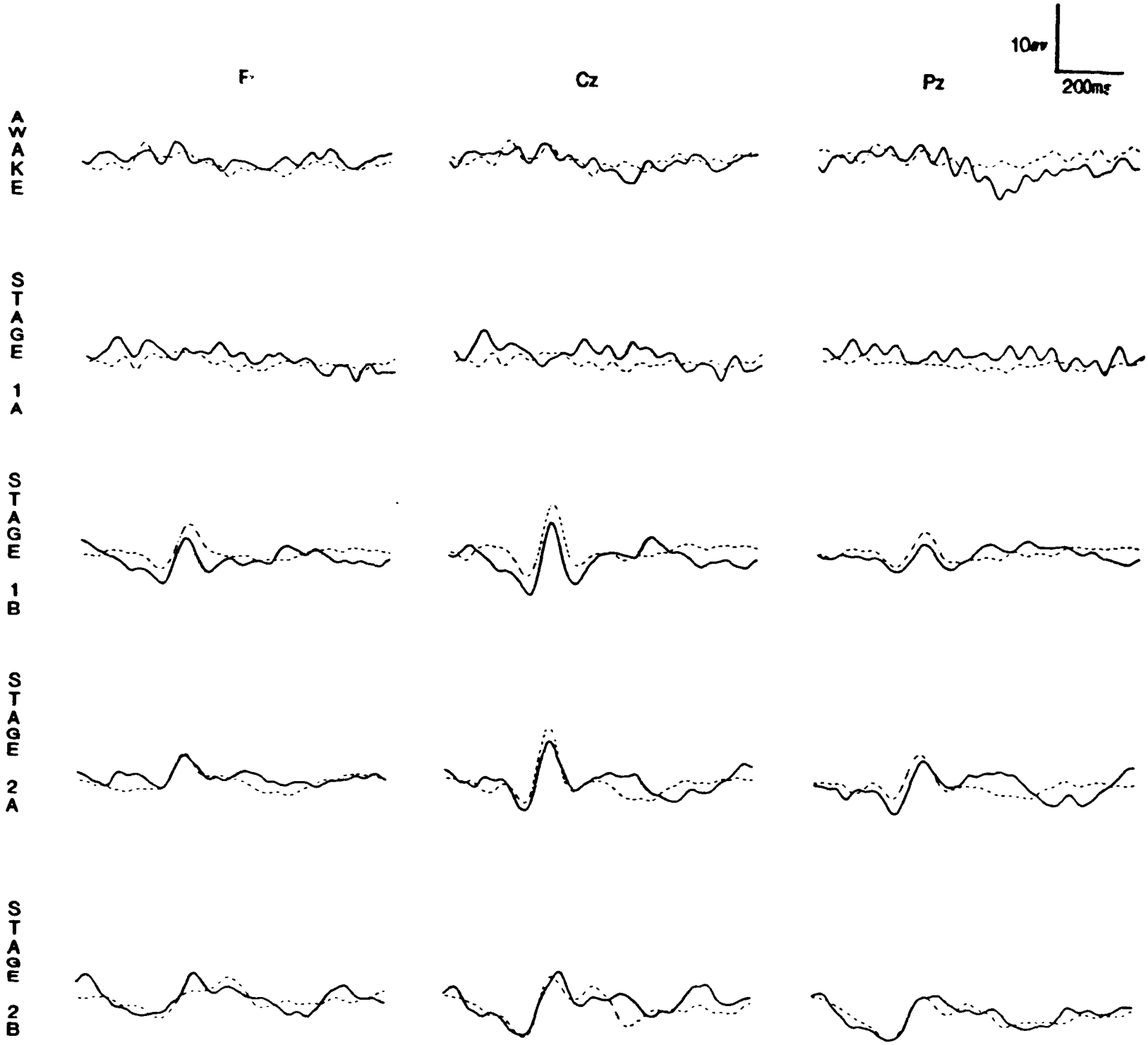
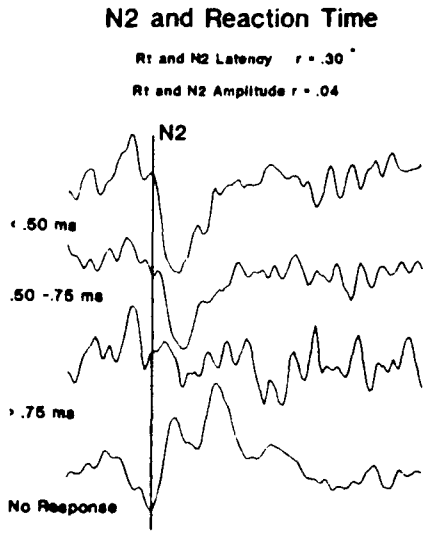


Figure 6 - Grand averages ERPs for control subjects

ERPs and Reaction Time

	Awake	1A	1B	2A	2B
Reaction time	0.78	1.08	1.34	1.42	1.59
N2 Latency	280	288	292	300	306
N2 Amplitude	-6.5	-8.3	-16.2	-24.3	-22.4
P3 Latency	434	430	435	456	453
P3 Amplitude	16.1	15.2	14.5	14.8	15.2



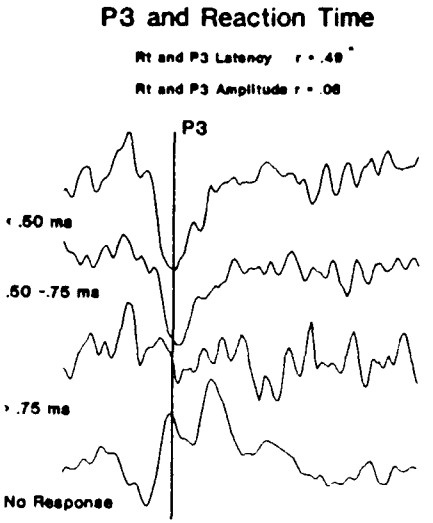


Figure 7 - ERP/RT correspondance

References for Experiment 6

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7.0 CONCLUSIONS

Considerable progress has been made towards meeting the objectives of this project. Especially significant was the acquistion of hardware and development of general purpose software to extend our ability to measure, analyze, and interpret characteristics of ERPs. We now possess the ability to collect, score, and interpret ERP and performance data using microprocessor-based systems that could if necessary be made portable. We believe this to be an especially significant achievement as ERP data are, in most labs, collected using either expensive and fixed-location minicomputers or microprocessors that have been equipped and programmed for clinical purposes. Clinical machines are limited in most cases to signal averaging and do not permit full exploration of data. The program that we have developed (called "EVAL" permits extensive data selection (including individual sweeps), data manipulation (artifact correction, filtering), and data analysis (averaging, scoring, etc). The EVAL program is user friendly and written in "C".

We have also further developed our battery of performance measures. We have acquired a recent version of the Performance Assessment Battery. We have also developed a response apparatus that permits the study of reaction times with a response (finger lift) that can be made quickly and easily, and with a minimum of effort. The subject's hand is strapped to the apparatus with the response "finger" continuously held in a "response ready" position. Also the apparatus is constructed so that the subjects can assume any body position, standing, sitting, supine, prone, etc and move their hand relatively freely without interfering with the ability to make a response.

In our earlier research contract funded by the US Army, the ERP measures of primary interest were the latencies and amplitudes of the auditory evoked potential components. The potentials were collected using an "oddball" paradigm which required the subjects to attend and selectively respond to a Bernoulli series of high and low pitched tone. We were encouraged by the finding that certain of these measures were correlated with circadian variation in performance and with the deterioration of performance with sleep loss. The research completed during the first half of the current contract period has to a degree replicated and has extended these findings. We have explored the findings using equipment and protocols that have provided for a more precise examination of the relationships and the underlying processes.

Experiments 1, 2, 3, and 5 are important in that they explore basic processes that might explain ERP variation in performance settings. All further our understanding of the important role of habituation in ERP variation. Experiment 1 suggests that changes in arousal level does not provide a sufficient explanation of P300 amplitude variation during and extended and "boring" test session and that attention may be the major factor. It may be important that variation in P300

amplitude was found even though accuracy of responding did not vary. It would be interesting to determine whether the reductions in P300 amplitude during the simple RT task are associated with "readiness" to respond to rare or unexpected stimulus events. The results of Experiment 2 suggest that ERPs may be components of an "orienting response" but are either more sensitive than peripheral measures of orienting or reflect other cognitive mechanisms. Experiment 3 provides considerable information about ERP changes during Pavlovian conditioning. Arousal again was explored as an explanatory factor. Variation of arousal may not be a sufficient condition for ERP variation but evidence was obtained to suggest that the cognitive processes reflected by P300 may be altered by increases in arousal associated with aversive events. The finding that the ERPs of the learners differed from those of nonlearners is a demonstration that ERPs are related to individual differences in performance.

Each of the experiments, especially Experiments 4-6 show that ERPs are related to variations in performance. Finding ERP correlates of time-of-day and ultradian variation in performances 1) establishes the sensitivity of ERP measures, 2) provides information about the processes underlying such performance variation, and 3) suggests that ERPs might be useful as predictors of performance readiness. With regard to the latter, the relationship between behavior and the negative ERP potentials, N1 and N2, should receive special attention as they can be obtained with a procedure which does not require a response or even the attention of the subject. This would enable use of the measure with subjects who because of work demands, motivation levels, etc are unable or unwilling to devote attention to an experimental task.

Experiment 6 was basically a pilot study exploring the feasibility of studying the relationships among sleepiness, performance, and ERP variables during the wake/sleep transition. Long term sleep deprivation studies require an enormous expenditure of time and resources and mid-experiment changes in protocol suggested by data or experience are usually not practical. The wake/sleep transition, however, provides the opportunity to study ERP/peformance relationships at levels of arousal from alert wakefulness to full sleep in a short period of time. The wake/sleep transition experiment addresses some of the objectives of this contract, i.e., it allows us to determine 1) whether ERPs change systematically as a consequence of sleepiness and sleep onset; 2) whether ERP changes are correlated with performance changes; and 3) whether ERPs are correlated with individual differences in performance. Most importantly, the wake/sleep transition experiment allows us to quickly and efficiently address some of the technical objectives of the contract such as 1) assessing the the role of latency variability in reduced component amplitudes, 2) determining whether collecting ERPs from multiple sites will enable us to more clearly describe neurophysiological changes, 3) establishing

whether alternative means of data reduction such as principle component analysis will provide a clearer description of the relationships, 5) exploring whether the findings obtained with auditory potentials will generalize to other stimulus modalities, and 6) exploring whether the several measures of evoked potentials are selectively related to different aspects of performance (response speed, memory retrieval, etc.). Preliminary results from the several wake/sleep experiments initiated but not yet completed support these claims and have convinced us that these experiments will greatly increase the yield of the long term deprivation experiments planned.

The completed experiments and the experiments in progress are bringing us closer to our contract goals of gaining a more complete understanding of the effects of prolonged stress on evoked-potential components and their relationship with performance and showing that a neurophysiological test based on ERP measures can be used a) to predict changes in performance resulting from exposure to prolonged stress and b) to distinguish between those individuals who will perform well and those who will perform poorly.